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(54) Title: HEPATITIS B VIRAL VARIANTS WITH REDUSED SUSCEPTIBILITY TO NUCLEOSIDE ANALOGS AND USES THEREOF

(57) Abstract: The present invention relates generally to viral variants exhibiting reduced sensitivity to particular agents and/or reduced interactivity with immunological reagents. More particularly, the present invention is directed to hepatitis B virus (HBV) variants exhibiting complete or partial resistance to nucleoside or nucleotide analogs and/or reduced interactivity with antibodies to viral surface components including reduced sensitivity to these antibodies. The present invention further contemplates assays for detecting such viral variants, which assays are useful in monitoring anti-viral therapeutic regimens and in developing new or modified vaccines directed against viral agents and in particular HBV variants. The present invention also contemplates the use of the viral variants to screen for and/or develop or design agents capable of inhibiting infection, replication and/or release of the virus.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
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PCT/AU03/00432	11 April 2003	12 April 2002

Applicant

MELBOURNE HEALTH et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

HEPATITIS B VIRAL VARIANTS WITH REDUCED SUSCEPTIBILITY TO NUCLEOSIDE ANALOGS AND USES THEREOF

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[X] None of the figures

VIRAL VARIANTS, DETECTION AND USE

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to viral variants exhibiting reduced sensitivity to particular agents and/or reduced interactivity with immunological reagents. More particularly, the present invention is directed to hepatitis B virus (HBV) variants exhibiting complete or partial resistance to nucleoside or nucleotide analogs and/or reduced interactivity with antibodies to viral surface components including reduced sensitivity to these antibodies. The present invention further contemplates assays for detecting such viral variants, which assays are useful in monitoring anti-viral therapeutic regimens and in developing new or modified vaccines directed against viral agents and in particular HBV variants. The present invention also contemplates the use of the viral variants to screen for and/or develop or design agents capable of inhibiting infection, replication and/or release of the virus.

DESCRIPTION OF THE PRIOR ART

Bibliographic details of the publications referred to in this specification are also collected at the end of the description.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in any country.

Hepatitis B virus (HBV) can cause debilitating disease conditions and can lead to acute liver failure. HBV is a DNA virus which replicates *via* an RNA intermediate and utilizes reverse transcription in its replication strategy (Summers and Mason, *Cell 29:* 403-415, 1982). The HBV genome is of a complex nature having a partially double-stranded DNA

structure with overlapping open reading frames encoding surface, core, polymerase and X genes. The complex nature of the HBV genome is represented in Figure 1. The polymerase consists of four functional regions, the terminal protein (TP), spacer, reverse transcriptase (rt) and ribonuclease (RNAse).

The polymerase gene of HBV overlaps the envelope gene, mutations in the catalytic domain of the polymerase gene can also affect the nucleotide and the deduced amino acid sequence of the envelope protein and *vice versa*. In particular, the genetic sequence for the neutralization domain of HBV known as the 'a' determinant, which is found within the HBsAg and located between amino acids 99 and 169, actually overlaps the major catalytic regions of the viral polymerase protein and in particular domains A and B.

The presence of an HBV DNA polymerase has led to the proposition that nucleoside or nucleotide analogs could act as effective anti-viral agents. Examples of nucleoside analogs currently being tested are penciclovir and its oral form (FCV) [Vere Hodge, *Antiviral Chem Chemother 4:* 67-84, 1993; Boyd *et al., Antiviral Chem Chemother. 32:* 358-363, 1987; Kruger *et al., Hepatology 22:* 219A, 1994; Main *et al., J. Viral Hepatitis 3:* 211-215, 1996], Lamivudine[(-)-β-2'-deoxy-3'-thiacytidine]; (3TC or LMV) [Severini *et al., Antimicrobial Agents Chemother. 39:* 430-435, 1995; Dienstag *et al., New England J Med 333:* 1657-1661, 1995]. New nucleoside or nucleotide analogs which have already progressed to clinical trials include the pyrimidines Emtricitabine, ((-)-β-L-2'-3'-dideoxy-5-fluoro-3'-thiacydidine; FTC), the 5-fluoro derivative of 3TC, and Clevudine (1-(2-fluoro-5-methyl-β-L-arabino-furanosyl) uracil; L-FMAU), a thymidine analog. Like 3TC, these are pyrimidine derivatives with an unnatural "L"- configuration. Several purine derivatives have also progressed to clinical trials; they include Entecavir (BMS-200, 475; ETV), a carbocyclic deoxyguanosine analog, diaminopurine dioxolane (DAPD), an oral pro-drug for dioxolane guanine ((-)-β-D-2-aminopurine dioxolane; DXG) and Adefovir dipivoxil, an oral prodrug for the acyclic deoxyadenosine monophosphate nucleoside analog Adefovir (9-[phosphonyl-methoxyethyl]-adenine; PMEA). Other drugs in pre-clincial and clinical trials include include FLG [Medivir], ACH-126,443 (L-d4C) [Archillion Pharmaceuticals], ICN 2001-3 (ICN) and Racivir (RCV) [Pharmassett].

Whilst these agents are highly effective in inhibiting HBV DNA synthesis, there is the potential for resistant mutants of HBV to emerge during long term antiviral chemotherapy. In patients on prolonged LMV therapy, key resistance mutations are selected in the rt domain within the polymerase at rtM204I/V +/- rtL180M as well as other mutations. The nomenclature used for the polymerase mutations is in accordance with that proposed by Stuyver *et al.*, 2001, *supra*. LMV is a nucleoside analog that has been approved for use against chronic HBV infection. LMV is a particularly potent inhibitor of HBV replication and reduces HBV DNA titres in the sera of chronically infected patients after orthotopic liver transplantation (OLT) by inhibiting viral DNA synthesis. LMV monotherapy seems unlikely to be able to control HBV replication in the longer term. This is because emergence of LMV-resistant strains of HBV seems almost inevitable during monotherapy.

Adefovir dipivoxil (ADV: formerly, bis-pom PMEA) is an orally available prodrug of the acyclic deoxyadenosine monophosphate analog adefovir (formerly, PMEA) (Figure 2). ADV is also a potent inhibitor of HBV replication and has recently been given FDA approval for use against chronic HBV infection. Adefovir dipivoxil differs from other agents in this class in that it is a nucleotide (*vs.* nucleoside) analog and as such bypasses the first phosphorylation reaction during drug activation. This step is often rate-limiting. Adefovir dipivoxil has demonstrated clinical activity against both wild-type and lamivudine-resistant strains of HBV and is currently in phase III clinical Testing (Gilson *et al., J Viral Hepat 6:* 387-395, 1999; Perrillo *et al., Hepatology 32:* 129-134, 2000; Peters *et al., Transplantation 68:* 1912-1914, 1999; Benhamou *et al., Lancet 358:* 718-723, 2001). During phase II studies a 30 mg daily dose of adefovir dipivoxil resulted in a mean 4 $\log_{10}$ decrease in viremia over 12 weeks (Heathcote *et al., Hepatology 28:* A620, 1998).

ADV is a substituted acyclic nucleoside phosphonate. This class of compounds also includes tenofovir disoproxil fumarate (also referred to as tenofovir DF, or tenofovir, or (TFV) or 9-R-(2-phosphonomethoxypropyl)adenine (PMPA) and is marketed as Viread by Gilead sciences).

TFV has antiviral activity against both HBV and HIV (Ying *et al., J Viral Hepat. 7(2):* 161-165, 2000; Ying *et al., J. Viral Hepat. 7(1):* 79-83, 2000; Suo *et al., J Biol Chem. 273(42):* 27250-27258. 1998).

FTC has activity against HBV and HIV (Frick *et al., Antimicrob Agents Chemother 37:* 2285-2292, 1993).

Nucleoside or nucleotide analog therapy may be administered as monotherapy or combination therapy where two or more nucleoside or nucleotide analogs may be administered. The nucleoside or nucleotide analogs may also be administered in combination with other antiviral agents such as interferon or hepatitis B immunoglobulin (HBIG).

There is a need to monitor for the emergence of nucleoside/nucleotide-analog- or antibody-resistant strains of HBV and to develop diagnostic protocols to detect these resistant viruses and/or to use them to screen for and/or develop or design agents having properties making them useful as anti-viral agents. Defective forms of these resistant strains or antigenic components therefrom are also proposed to be useful in the development of therapeutic vaccine compositions as are antibodies directed to viral surface components.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

Specific mutations in an amino acid sequence are represented herein as "Xaa_1nXaa_2" where Xaa_1 is the original amino acid residue before mutation, n is the residue number and Xaa_2 is the mutant amino acid. The abbreviation "Xaa" may be the three letter or single letter (i.e. "X") code. An "rt" before "Xaa_1nXaa_2" means "reverse transcriptase". An "s" means an envelope gene. The amino acid residues for HBV DNA polymerase are numbered with the residue methionine in the motif Tyr Met Asp Asp (YMDD) being residue number 204 (Stuyver *et al., Hepatology 33:* 751-757, 2001). The amino acid residues for hepatitis B virus surface antigen are number according to Norder *et al.* (*J. Gen. Virol. 74:* 341-1348, 1993). Both single and three letter abbreviations are used to define amino acid residues and these are summarized in Table 2.

In accordance with the present invention, the selection of HBV variants is identified in patients (Patient A, C and D) with chronic HBV infection treated with ADV and liver transplant patients (Patients B and E) treated with both ADV and LMV post-OLT or ADV post-transplant. HBV variants from Patients F, G and H were also identified following similar treatments. Variants of HBV are identified during ADV or combination ADV and LMV treatment with mutations in the HBV DNA polymerase gene which reduce the sensitivity of HBV to this nucleoside analog. Consequently, HBV rt variants are contemplated which are resistant to, or which exhibit reduced sensitivity to, ADV,

LMV,TFV, FTC, ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agentsor combinations thereof . Corresponding mutations in the surface antigen also occur. The identification of these HBV variants is important for the development of assays to monitor ADV, LMV, FTC and/or TFV resistance and/or resistance to other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof and to screen for agents which are useful as alternative therapeutic agents.

Reference herein to "anti-HBV agents" includes nucleoside and nucleotide analogs as well as immunological reagents (e.g. antibodies to HBV surface components) and chemical, proteinaceous and nucleic acid agents which inhibit or otherwise interfere with viral replication, maintenance, infection, assembly or release.

The detection of such HBV variants is particularly important in the management of therapeutic protocols including the selection of appropriate agents for treating HBV infection. The method of this aspect of the present invention is predicated in part on monitoring the development in a subject of an increased HBV load in the presence of a nucleoside or nucleotide analog or other anti-HBV agents or combinations thereof. The clinician is then able to modify an existing treatment protocol or select an appropriate treatment protocol accordingly.

Accordingly, one aspect of the present invention is directed to an isolated HBV variant comprising a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to the DNA polymerase and which exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof. The variant HBV

comprises a mutation in an overlapping open reading frame in its genome in a region defined by one or more of domains F and G and domain A through to E of HBV DNA polymerase.

Another aspect of the present invention provides an isolated HBV variant comprising a nucleotide mutation in the S gene resulting in at least one amino acid addition, substitution and/or deletion to the surface antigen and which exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, or ADV and FTC and LMV and TFV, ADV and LMV and FTC, and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof.

Useful mutants in the rt region include, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; and in yet another embodiment, rtH90D and rtL/F108L; and in still a further embodiment, rtL157L/M, rtA181V and rtV207I and in yet a further embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; and in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H or a combination thereof or an equivalent mutation.

Other HBV variants are also contemplated with mutations in rt at rtK32, rtN33, rtP34, rtH35 and rtT37 (these are upstream of the F domain of the DNA polymerase), rtP59, rtK60, rtF61, rtA62 and rtV63 (these are located between the F and A domains), rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91 (these are located within the A domain and the region immediately prior to and following), rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184 (these are located in the B domain), rtM204 and rtY203 (these

are located in the C domain), rt235, rt236, rt237, rt238 and rt239 (these are located in the D domain) and rt247, rt248, rt249, rt250 and rt251 (these are located in the E domain) or a combination thereof or an equivalent mutation.

Useful mutants are provided below (see also Tables 16 and 17):

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion.

Reference above to "deletion" means that the first mentioned amino acid before the residue number has been deleted.

Useful mutations in the S gene include, in one embodiment, sP120T, sM125T and sT127A; in another embodiment, T118R, sM133T, sF134V sI195M, sS207R and sY225Y/C; in a further embodiment, sS126T, sM133L/M, sS143S/T, sD144A sG145A and sW172Stop; in yet a further embodiment, sN40S, sC69 Stop, sM75I, sL88P, sT118A, sW182stop, sW196L, sY206H and sY225F; and in yet another embodiment, sI81M and sP214Q; and in still another embodiment, sF83S, sL173F and sW199L; and in still yet another embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; and in yet another embodiment, sC69Stop/C, sC76Y sI110V/I, sY134N,

sW172Stop/W, sW196Stop and sS207R or a combination thereof or an equivalent mutation.

The present invention further contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof by isolating DNA or corresponding mRNA from the HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G and domains A through to E or a region proximal thereto of the DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. The presence of such a mutation is an indication of the likelihood of resistance to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The present invention also provides a composition comprising a variant HBV resistant to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, or ADV and FTC and LMV and TFV, ADV and LMV and FTC, and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof or an HBV surface antigen from the variant HBV or a recombinant or

derivative form thereof or its chemical equivalent and one or more pharmaceutically acceptable carriers and/or diluents.

Yet another aspect of the present invention provides a use of the aforementioned composition or a variant HBV comprising a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to the DNA polymerase and a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof in the manufacture of a medicament for the treatment and/or prophylaxis of hepatitis B virus infection.

The present invention also contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents or by isolating DNA or corresponding mRNA from the HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase wherein the presence of the following mutations in the rt region: in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment, rtH90D and rtL/F108L, in even yet another embodiment, rtL157L/M, rtA181V and rtV207I; in still yet another embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H; in a further embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37; in yet another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63; in still another embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91; in even yet another embodiment, rtP177,

rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in still yet another embodiment, rtM204 and rtY203; in another embodiment, rt235, rt236, rt237, rt238 and rt239; in a further embodiment, rt247, rt248, rt249, rt250 and rt251 or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV,TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Still a further methodology comprises screening for a mutation in the nucleotide sequence encoding the envelope genes (s) wherein the presence of the following mutations in the S gene: in one embodiment, sP120T, sM125T and sT127A; in another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in a further embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop in yet another embodiment, sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182Stop, sW196L, sY206H and sY225F; in still yet another embodiment, s181M and sP214Q; in another embodiment, sF83S, sL173F and sW199L; in a further aspect, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in a further embodiment, sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV,TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Preferably, the variants are in an isolated form such that they have undergone at least one purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also contemplates infectious molecular clones comprising the genome or parts thereof from a

variant HBV. The detection of HBV or its components in cells, cell lysates, cultured supernatant fluid and bodily fluid may be by any convenient means including any nucleic acid-based detection means, for example, by nucleic acid hybridization techniques or *via* one or more polymerase chain reactions (PCRs). The term "bodily fluid" includes any fluid derived from the blood, lymph, tissue or organ systems including serum, whole blood, biopsy and biopsy fluid, organ explants and organ suspension such as liver suspensions.

Another aspect of the present invention is directed to a variant HBV comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to a surface antigen from a reference or wild type HBV and wherein an antibody generated to the reference or wild type surface antigen exhibits an altered immunological profile relative to the HBV variant. One altered profile includes a reduced capacity for neutralizing the HBV. More particularly, the surface antigen of the variant HBV exhibits an altered immunological profile compared to a pre-treatment HBV where the variant HBV is selected for by a nucleoside or nucleotide analog or other anti-HBV agents of the HBV DNA polymerase. The variant HBV of this aspect of the invention may also comprise a nucleotide sequence comprising a single or multiple nucleotide substitution, addition and/or deletion compared to a pre-treatment HBV.

The present invention extends to an isolated HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof corresponding to the variant HBV. Generally, the HBsAg or its recombinant or derivative form or its chemical equivalent comprises an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to an HBsAg from a reference HBV and wherein an antibody directed to a reference HBV exhibits an altered immunological profile to an HBV carrying said variant HBsAg. In one embodiment, the altered immunological profile comprises a reduction in the ability to neutralize the variant HBV.

Another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV by generating a genetic construct comprising

a replication competent-effective amount of the genome from the HBV contained in a plasmid vector and then transfecting said cells with said construct, contacting the cells, before, during and/or after transfection, with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agents; and the subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent. In a preferred embodiment, the plasmid vector in a baculovirus vector and the method comprises generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct, contacting the cells, before, during and/or after infection, with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent and then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In connection with these methods, the plasmid vector may include genes encoding part or all of other viral vectors such as baculovirus vectors or adenovirus vectors (see Ren and Nassal, *J. Virol. 75(3):* 1104-1116, 2001).

In an alternative embodiment, the method comprises generating a continuous cell line comprising an infectious copy of the genome of the HBV in a replication competent effective amount such that said infectious HBV genome is stably integrated into said continuous cell line such as but not limited to the 2.2.15 or AD cell line, contacting the cells with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to the agent and then subjecting the cells,

cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In an alternative embodiment, the present invention also contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV polymerase in an *in vitro* polymerase assay. The HBV polymerase activity can be examined using established assays (Gaillard *et al.*, *Antimicrob Agents Chemother. 46(4)*: 1005-1013, 2002; Xiong *et al.*, *Hepatology. 28(6)*: 1669-73, 1998). The HBV polymerase may be a wild-type or reference HBV polymerase or mutant HBV polymerase.

The identification of viral variants enables the production of vaccines comprising particular recombinant viral components such as polymerases or envelope genes PreS1, PreS2, S encoding for L, M, S proteins as well as therapeutic vaccines comprising defective HBV variants. Rational drug design may also be employed to identify or generate therapeutic molecules capable of interacting with a polymerase or or envelope genes PreS1, PreS2, S encoding for L, M, S proteins or other component of the HBV. Such drugs may also have diagnostic potential. In addition, defective HBV variants may also be used as therapeutic compositions to generate an immune response against the same, similar or homologous viruses. Alternatively, antibodies generated to the HBV variants or surface components thereof may be used in passive immunization of subjects against infection by HBV variants or similar or homologous viruses. Furthermore, agents such as nucleoside or nucleotide analogs, RNAi or siRNA molecules, antisense or sense oligonucleotides, chemical or proteinaceous molecules having an ability to down-regulate the activity of a component of HBV and inhibit replication, maintenance, infection, assembly or release are contemplated by the present invention.

A summary of the abbreviations used throughout the subject specification are provided in Table 3.

A summary of sequence identifiers used throughout the subject specification is provided in Table 1.

TABLE 1

Summary of sequence identifiers

SEQUENCE ID NO:	DESCRIPTION
1	Formula I
2	Formula II
3	OS1 primer
4	TTA3 primer
5	JM primer
6	TTA4 primer
7	OS2 primer
8	sense primer
9	antisense primer
10	internal regions primer
11	internal regions primer
12	PC1 forward primer
13	PC2 reverse primer
14	HBV-specific molecular beacon primer
15	ILA 1 F, A-E (Figure 4)
16	ILA 2 F, A-E (Figure 4)
17	ILA 3 F, A-E (Figure 4)
18	ILA 4 F, A-E (Figure 4)
19	Pol Trans Pre 1 (Figure 5)
20	Pol Trans 2 (Figure 5)
21	Pol Trans 3 (Figure 5)
22	Pol Trans 4 (Figure 5)
23	HBsAg Trans of Pre 1 (Figure 6)

SEQUENCE ID NO:	DESCRIPTION
24	HBsAg Trans of 2 (Figure 6)
25	HBsAg Trans of 3 (Figure 6)
26	HBsAg Trans of 4(Figure 6)
27	S0 (Figure 7)
28	S6 (Figure 7)
29	S8 (Figure 7)
30	S12 (Figure 7)
31	S15 (Figure 7)
32	Pol Trans S0 (Figure 8)
33	Pol Trans S6 (Figure 8)
34	Pol Trans S8 (Figure 8)
35	Pol Trans S12 (Figure 8)
36	Pol Trans S15 (Figure 8)
37	HBsAg Trans of S0 (Figure 9)
38	HBsAg Trans of S6 (Figure 9)
39	HBsAg Trans of S8 (Figure 9)
40	HBsAg Trans of S12 (Figure 9)
41	HBsAg Trans of S15 (Figure 9)
42	Nucleotide sequence Patient C (Figure 10)
43	POL Trans of Patient C (Figure 11)
44	HBsAg Trans of Patient C (Figure 12)
45	Nucleotide sequence of Patient D (Figure 13)
46	Pol Trans of Patient D (Figure 14)
47	HBsAg Trans of Patient D (Figure 15)
48	Nucleotide sequence of Patient E (Figure 16)
49	Pol Trans of Patient E (Figure 17)
50	HBsAg Trans of Patient E (Figure 18)
51	Nucleotide sequence of Patient F (Figure 20)
52	Deduced sequence of DNA polymerase of Patient F (Figure 21)

SEQUENCE ID NO:	DESCRIPTION
53	HBsAg Trans of Patient F (Figure 22)
54	Nucleotide sequence of Patient G (Figure 23)
55	Deduced sequence of DNA polymerase of Patient G (Figure 24)
56	HBsAg Trans of Patient G (Figure 25)
57	Nucleotide sequence of Patient H (Figure 26)
58	Deduced sequence of DNA polymerase of Patient H (Figure 27)
59	HBsAg Trans of Patient H (Figure 28)

TABLE 2

Single and three letter amino acid abbreviations

Amino Acid	Three-letter Abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	The	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

A list of abbreviations used throughout the subject specification are provided in Table 3.

TABLE 3

Abbreviations

ABBREVIATION	DESCRIPTION
3TC	(LMV); (-)-β-2'-deoxy-3'-thiacytidine
ADV	adefovir dipivoxil
DAPD	diaminopurine dioxalone
DXG	dioxolane guanine
ETV	entecavir
FAM	famciclovir
FCV	famciclovir
FTC	emtricitabine
HBIG	hepatitis B immunoglobulin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
LMV	lamividuine
PMEA	9-[phosphonyl-methoxyethyl]-adenine; adefovir
PMPA	9-R-(2-phosphonomethoxypropyl)adenine
RNase	ribonuclease
rt ("rt" before "Xaa_1nXaa_2" means reverse transcriptase)	reverse transcriptase
s (as used in a mutation, e.g. sF134V)	envelope gene
TFV	tenofovir disoproxil fumarate
YMDD	Tyr Met Asp Asp-a motif in the polymerase protein; where the Met residue is designated residue number 204 of the reverse transcriptase

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing the partially double stranded DNA HBV genome showing the overlapping open reading frames encoding surface (S), core (C), polymerase (P) and X gene.

Figure 2 is a diagrammatic representation of the chemical structure of ADV.

Figure 3 is a diagrammatic representation of a computer system for determining the potency value (P_A) of a variant HBV.

Figure 4 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient A during ADV treatment.

Figure 5 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient A during ADV therapy.

Figure 6 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient A during ADV therapy.

Figure 7 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient B during ADV and LMV treatment.

Figure 8 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient B during ADV and LMV therapy.

Figure 9 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient B during ADV and LMV therapy.

Figure 10 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient C during ADV treatment.

Figure 11 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient C during ADV therapy.

Figure 12 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient C during ADV therapy.

Figure 13 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient D during ADV treatment.

Figure 14 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient D during ADV therapy.

Figure 15 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient D during ADV therapy.

Figure 16 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient E during ADV treatment.

Figure 17 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient E during ADV therapy.

Figure 18 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient E during ADV therapy.

Figure 19 is a diagrammatic representation of a system used to carry out the instructions encoded by the storage medium.

Figure 20 is a representation showing the nucleotide sequence of envelope/rt region of an HBV isolated from Patient F having ADV therapy.

Figure 21 is a representation showing the deduced amino acid sequence of DNA polymerase encoded by the nucleotide sequence shown in Figure 20.

Figure 22 is a representation showing the deduced amino acid sequence of HBsAg encoded by the nucleotide sequence shown in Figure 20.

Figure 23 is a representation showing the nucleotide sequence of envelope/rt region of an HBV isolated from Patient G having ADV therapy.

Figure 24 is a representation showing the deduced amino acid sequence of DNA polymerase encoded by the nucleotide sequence shown in Figure 23.

Figure 25 is a representation showing the deduced amino acid sequence of HBsAg encoded by the nucleotide sequence shown in Figure 23.

Figure 26 is a representation showing the nucleotide sequence of envelope/rt region of an HBV isolated from Patient H having ADV therapy.

Figure 27 is a representation showing the deduced amino acid sequence of DNA polymerase encoded by the nucleotide sequence shown in Figure 26.

Figure 28 is a representation showing the deduced amino acid sequence of HBsAg encoded by the nucleotide sequence shown in Figure 26.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated in part on the identification and isolation of nucleoside or nucleotide analog-resistant variants of HBV following treatment of patients with either ADV or LMV or more particularly ADV and LMV, or optionally other nucleoside analogs or nucleotide analogs or other anti-HBV agents such as TFV or FTC. In particular, ADV or ADV and LMV treated patients gave rise to variants of HBV exhibiting decreased or reduced sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV. . Reference herein to "decreased" or "reduced" in relation to sensitivity to ADV and/or LMV and/or FTC and/or TFV includes and encompasses a complete or substantial resistance to the nucleoside or nucleotide analog or other anti-HBV agents as well as partial resistance and includes a replication rate or replication efficiency which is more than a wild-type in the presence of a nucleoside or nucleotide analog or other anti-HBV agents. In one aspect, this is conveniently measured by an increase in viral load during treatment, or alternatively, there is no substantial decrease in HBV DNA viral load from pre-treatment HBV DNA levels during treatment (i.e., non-response to treatment).

Before describing the present invention in detail, it is to be understood that unless otherwise indicated, the subject invention is not limited to specific formulations of components, manufacturing methods, dosage regimens, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleoside or nucleotide analog" includes a single analog, as well as two or more analogs; reference to "an HBV variant" includes reference to two or more HBV variants; and so forth.

In describing and claiming the present invention, the following terminology is used in accordance with the definitions set forth below.

The terms "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably herein to refer to a chemical compound that induces a desired effect such as inhibit viral replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc.

The present invention contemplates, therefore, compounds useful in inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Reference to an "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" such as ADV, LMV, FTC and/or TFV includes combinations of two or more actives such as ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV. A "combination" also includes a two-part or more such as a multi-part anti-HBV therapeutic composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation.

The terms "effective amount" and "therapeutically effective amount" of an agent as used herein mean a sufficient amount of the agent to provide the desired therapeutic or physiological effect of inhibiting HBV replication, infection, maintenance, assembly

and/or the function of an enzyme such as HBV DNA polymerase. Furthermore, an "effective HBV-inhibiting amount" or "effective symptom-ameloriating amount" of an agent is a sufficient amount of the agent to directly or indirectly inhibit replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

By "pharmaceutically acceptable" carrier, excipient or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emusifying agents, pH buffering agents, preservatives, and the like.

Similarly, a "pharmacologically acceptable" salt, ester, emide, prodrug or derivative of a compound as provided herein is a salt, ester, amide, prodrug or derivative that this not biologically or otherwise undesirable.

The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms and/or their underlying cause, and improvement or remediation of damage in relation to HBV infection. Thus, for example, "treating" a patient involves prevention of HBV infection as well as treatment of a clinically HBV symptomatic individual by inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Thus, for example, the

present method of "treating" a patient with HBV infection or with a propensity for one to develop encompasses both prevention of HBV infection as well as treating HBV infection or symptoms thereof. In any event, the present invention contemplates the treatment or prophylaxis of HBV infection.

"Patient" as used herein refers to an animal, preferably a mammal and more preferably a primate including a lower primate and even more preferably, a human who can benefit from the formulations and methods of the present invention. A patient regardless of whether a human or non-human animal may be referred to as an individual, subject, animal, host or recipient. The compounds and methods of the present invention have applications in human medicine, veterinary medicine as well as in general, domestic or wild animal husbandry. For convenience, an "animal" includes an avian species such as a poultry bird (including ducks, chicken, turkeys and geese), an aviary bird or game bird. The condition in a non-human animal may not be a naturally occurring HBV infection but HBV-like infection may be induced.

As indicated above, the preferred animals are humans, non-human primates such as marmossets, baboons, orangatangs, lower primates such as tupia, livestock animals, laboratory test animals, companion animals or captive wild animals. A human is the most preferred target. However, non-human animal models may be used.

Examples of laboratory test animals include mice, rats, rabbits, guinea pigs and hamsters. Rabbits and rodent animals, such as rats and mice, provide a convenient test system or animal model as do primates and lower primates. Livestock animals include sheep, cows, pigs, goats, horses and donkeys. Non-mammalian animals such as avian species, zebrafish, amphibians (including cane toads) and *Drosophila* species such as *Drosophila melanogaster* are also contemplated. Instead of a live animal model, a test system may also comprise a tissue culture system.

Accordingly, one aspect of the present invention is directed to an isolated HBV variant wherein said variant comprises a nucleotide mutation in a gene encoding a DNA

polymerase resulting in at least one amino acid addition, substitution and/or deletion to said DNA polymerase and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, or ADV and LMV and FTC, ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

HBV is a member of the Hepdnaviridae that includes also avian hepatitis viruses such as Duck hepatitis B virus (DHBV) and hepatitis viruses from mammals such as woodchuck hepatitis virus (WHV). These viruses have similarity to HBV and may be used in *in vitro* and *in vivo* or animal model systems to investigate the equivalent HBV mutants and anti-viral sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,

An "anti-HBV agent" includes a nucleoside or nucleotide analog, protein, chemical compound, RNA or DNA or RNAi or siRNA oligonucleotide.

Preferably, the decreased sensitivity is in respect of ADV. Alternatively, the decreased sensitivity is in respect of LMV. Alternatively, the decreased sensitivity is in respect of TFV. Alternatively, the decreased sensitivity is in respect of FTC. Alternatively, the decreased sensitivity is in respect of ADV and LMV. Alternatively, the decreased sensitivity is in respect of ADV and TFV. Alternatively, the decreased sensitivity is in respect of LMV and TFV. Alternatively, the decreased sensivity is in respect of ADV and FTC. Alternatively, the decreased sensitivity is in respect to FTC and TFV. Alternatively, the decreased sensitvity is in respect of FTC and LMV. Alternatively, the decreased sensitivity is in respect of ADV and LMV and TFV. Alternatively, the decreased sensivity is in respect to ADVand TFV and FTC. Alternatively, the decreased sensivity is in respect to LMV and TFV and FTC. Alternatively, the decrease senvitity is in respect of ADV and

LMV and FTC. Alternartively, the decreased sensitvity is in respect of ADV and FTC and TFV and LMV.

Reference herein to "anti-HBV agents" includes nucleoside and nucleotide analogs as well as immunological reagents (e.g. antibodies to HBV surface components) and chemical, proteinaceous and nucleic acid agents which inhibit or otherwise interfere with viral replication, maintenance, infection, assembly or release. Reference herein to "nucleic acid" includes reference to a sense or antisense molecule, RNA or DNA, oligonucleotides and RNAi and siRNA molecules and complexes containing same.

In addition to a mutation in the gene encoding DNA polymerase, due to the overlapping nature of the HBV genome (Figure 1), a corresponding mutation may also occur in the gene encoding the S gene encoding the surface antigen (HBsAg) resulting in reduced interactivity of immunological reagents such as antibodies and immune cells to HBsAg. The reduction in the interactivity of immunological reagents to a viral surface component generally includes the absence of immunological memory to recognize or substantially recognize the viral surface component. The present invention extends, therefore, to an HBV variant exhibiting decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, and/or ADV and FTC and LMV and TFV or a reduced interactivity of an immunological reagent to HBsAg wherein the variant is selected for following ADV and/or LMV combination or sequential treatment. The term "sequential" in this respect means ADV followed by LMV and/or TFV, and /or FTC, LMV followed by ADV and/or TFV, and /or FTC, or multiple sequential administrations of each of ADV, LMV and/or TFV, and /or FTC.

A viral variant may, therefore, carry mutation only in the DNA polymerase gene or both in the DNA polymerase gene and the S gene. The term "mutation" is to be read in its broadest context and includes multiple mutations.

The present invention extends to a mutation and any domain of the HBV DNA polymerase and in particular regions F and G, and domains A through to E provided said mutation leads to decreased sensitivity to ADV and/ or LMV and/or TFV or combinations thereof. Regions F and G of the HBV DNA polymerase is defined by the amino acid sequence set forth in Formula I below [SEQ ID NO:1]:

FORMULA I

L, X_1, X_2, D, W, G, P, C, X_3, X_4, H, G, X_5, H, X_6, I, R, B_7, P, R, T, P, X_8, R, V, X_9, G, G, V, F, L, V, D, K, N, P, H, N, T, X_{10}, E, S, X_{11}, L, X_{12}, V, D, F, S, Q, F, S, R, G, X_{13}, X_{14}, X_{15}, V, S, W, P, K, F, A, V, P, N, L, X_{16}, S, L, T, N, L, L, S*

wherein:

X_1 is L, or R, or I
X_2 is E, or D
X_3 is T, or D, or A, or N, or Y
X_4 is E, or D
X_5 is E, or K, or Q
X_6 is H, or R, or N,
X_7 is I, or T
X_8 is A, or S
X_9 is T or R
X_{10} is A, or T, or S
X_{11} is R, or T
X_{12} is V, or G
X_{13} is S, or I, or T, or N, or V
X_{14} is T, or S, or H, or Y
X_{15} is R, or H, or K, or Q
X_{16} is Q, or P;

and wherein S* is designated as amino acid 74.

In this specification, reference is particularly made to the conserved regions of the DNA polymerase as defined by domains A to E. Regions A to E are defined by the amino acid sequence set forth in Formula II below [SEQ ID NO:2] (and in Australian Patent No. 734831):

FORMULA II

S X_1 L S W L S L D V S A A F Y H X_2 P L H P A A M P H L L X_3 G S S G L X_4 R Y V A R L S S X_5 S X_6 X_7 X N X_8 Q X_9 X_{10} X X X X X_{11} L H X_{12} X_{13} C S R X_{14} L Y V S L X_{15} L L Y X_{16} T X_{17} G X_{18} K L H L X_{19} X_{20} H P I X_{21} L G F R K X_{22} P M G X_{23} G L S P F L L A Q F T S A I X_{24} X_{25} X_{26} X_{27} X_{28} R A F X_{29} H C X_{30} X_{31} F X_{32} Y M* D D X_{33} V L G A X_{34} X_{35} X_{36} X_{37} H X_{38} E X_{39} L X_{40} X_{41} X_{42} X_{43} X_{44} X_{45} X_{46} L L X_{47} X_{48} G I H L N P X_{49} K T K R W G Y S L N F M G Y X_{50} I G

wherein:

X	is any amino acid
X_1	is N or D;
X_2	is I or P;
X_3	is I or V;
X_4	is S or D;
X_5	is T or N;
X_6	is R or N;
X_7	is N or I;
X_8	is N or Y or H;
X_9	is H or Y;
X_{10}	is G or R;
X_{11}	is D or N;
X_{12}	is D or N;

X_{13} is S or Y;
X_{14} is N or Q;
X_{15} is L or M;
X_{16} is K or Q;
X_{17} is Y or F;
X_{18} is R or W;
X_{19} is Y or L;
X_{20} is S or A;
X_{21} is I or V;
X_{22} is I or L;
X_{23} is V or G;
X_{24} is C or L;
X_{25} is A or S;
X_{26} is V or M;
X_{27} is V or T;
X_{28} is R or C;
X_{29} is F or P;
X_{30} is L or V;
X_{31} is A or V;
X_{32} is S or A;
X_{33} is V or L or M;
X_{34} is K or R;
X_{35} is S or T;
X_{36} is V or G;
X_{37} is Q or E;
X_{38} is L or S or R;
X_{39} is S or F;
X_{40} is F or Y;
X_{41} is T or A;
X_{42} is A or S;
X_{43} is V or I;

X_{44} is T or C;
X_{45} is N or S;
X_{46} is F or V;
X_{47} is S or D;
X_{48} is L or V;
X_{49} is N or Q;
X_{50} is V or I; and
M^* is amino acid 204;

and wherein the first S is designated as amino acid 75.

Preferably, the mutation results in an altered amino acid sequence in any one or more of domains F and G, and domains A through to E or regions proximal thereto of the HBV DNA polymerase.

Another aspect of the present invention provides an HBV variant comprising a mutation in an overlapping open reading frame in its genome wherein said mutation is in a region defined by one or more of domains F and G, and domains A through to E of HBV DNA polymerase and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents.

In a related embodiment, there is provided an HBV variant comprising a mutation in the nucleotide sequence encoding a DNA polymerase resulting in an amino acid addition, substitution and/or deletion in said DNA polymerase in one or more amino acids as set forth in Formula I [SEQ ID NO:1] and/or Formula II [SEQ ID NO:2]:

FORMULA I

L, X_1, X_2, D, W, G, P, C, X_3, X_4, H, G, X_5, H, X_6, I, R, X_7, P, R, T, P, X_8, R, V, X_9, G, G, V, F, L, V, D, K, N, P, H, N, T, X_{10}, E, S, X_{11}, L, X_{12}, V, D, F, S, Q, F, S, R, G, X_{13}, X_{14}, X_{15}, V, S, W, P, K, F, A, V, P, N, L, X_{16}, S, L, T, N, L, L, S*

wherein:

X_1 is L, or R, or I

X_2 is E, or D

X_3 is T, or D, or A, or N, or Y

X_4 is E, or D

X_5 is E, or K, or Q

X_6 is H, or R, or N,

X_7 is I, or T

X_8 is A, or S

X_9 is T or R

X_{10} is A, or T, or S

X_{11} is R, or T

X_{12} is V, or G

X_{13} is S, or I, or T, or N, or V

X_{14} is T, or S, or H, or Y

X_{15} is R, or H, or K, or Q

X_{16} is Q, or P;

and

FORMULA II

S X_1 L S W L S L D V S A A F Y H X_2 P L H P A A M P H L L X_3 G S S G L X_4 R Y V A R L S S X_5 S X_6 X_7 X N X_8 Q X_9 X_{10} X X X X_{11} L H X_{12} X_{13} C S R X_{14} L Y V S L X_{15}

L L Y X_{16} T X_{17} G X_{18} K L H L X_{19} X_{20} H P I X_{21} L G F R K X_{22} P M G X_{23} G L S P F L L A Q F T S A I X_{24} X_{25} X_{26} X_{27} X_{28} R A F X_{29} H C X_{30} X_{31} F X_{32} Y M* D D X_{33} V L G A X_{34} X_{35} X_{36} X_{37} H X_{38} E X_{39} L X_{40} X_{41} X_{42} X_{43} X_{44} X_{45} X_{46} L L X_{47} X_{48} G I H L N P X_{49} K T K R W G Y S L N F M G Y X_{50} I G

wherein:

X is any amino acid
X_1 is N or D;
X_2 is I or P;
X_3 is I or V;
X_4 is S or D;
X_5 is T or N;
X_6 is R or N;
X_7 is N or I;
X_8 is N or Y or H;
X_9 is H or Y;
X_{10} is G or R;
X_{11} is D or N;
X_{12} is D or N;
X_{13} is S or Y;
X_{14} is N or Q;
X_{15} is L or M;
X_{16} is K or Q;
X_{17} is Y or F;
X_{18} is R or W;
X_{19} is Y or L;
X_{20} is S or A;
X_{21} is I or V;
X_{22} is I or L;
X_{23} is V or G;

X_{24} is C or L;
X_{25} is A or S;
X_{26} is V or M;
X_{27} is V or T;
X_{28} is R or C;
X_{29} is F or P;
X_{30} is L or V;
X_{31} is A or V;
X_{32} is S or A;
X_{33} is V or L or M;
X_{34} is K or R;
X_{35} is S or T;
X_{36} is V or G;
X_{37} is Q or E;
X_{38} is L or S or R;
X_{39} is S or F;
X_{40} is F or Y;
X_{41} is T or A;
X_{42} is A or S;
X_{43} is V or I;
X_{44} is T or C;
X_{45} is N or S;
X_{46} is F or V;
X_{47} is S or D;
X_{48} is L or V;
X_{49} is N or Q;
X_{50} is V or I; and
M^* is amino acid 204;

and wherein S* in Formula I is designated as amino acid 74 and the first S in Formula II is designated as amino acid 75;

and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. Preferably, the decreased sensitivity is to ADV or to both ADV and LMV or to one or both of ADV and/or LMV and/or TFV and /or FTC.

Another preferred aspect of the present invention contemplates an HBV variant comprising a mutation in the nucleotide sequence encoding HBsAg resulting in an amino acid addition, substitution and/or deletion in said HBsAg in a region corresponding to the amino acid sequence set forth in Formulae I and II wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

More particularly, the present invention provides a variant HBV comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to a surface antigen from a reference or wild-type HBV and wherein an antibody generated to the reference or wild-type surface antigen exhibits reduced capacity for neutralizing said HBV variant, said variant selected by exposure of a subject to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The term "combination therapy" means that both combinations of ADV, LMV, FTC and/or TFV are co-administered in the same composition or simultaneously in separate compositions. The term "sequential therapy" means that the two agents are administered within seconds, minutes, hours, days or weeks of each other and in either order. Sequential therapy also encompasses completing a therapeutic course with one or other of ADV, LMV, FTC or TFV and then completing a second or third or subsequent therapeutic courses with the other of ADV, LMV, FTC or TFV.

Accordingly, another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even still another aspect of of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

A further aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure

of a subject to LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to TFV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC and LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile

compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

A further aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and

wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, FTC and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV, FTC and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Preferably, the variants are in isolated form such that they have undergone at least one purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also contemplates infectious molecular clones comprising the genome or parts thereof from a variant HBV. Furthermore, the present invention provides isolated components from the variant HBVs such as but not limited to an isolated HBsAg. Accordingly, the present invention provides an isolated HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

More particularly, yet another aspect of the present invention is directed to an isolated variant HBsAg or a recombinant or derivative form thereof or a chemical equivalent thereof wherein said HBsAg or its recombinant or derivative form or its chemical equivalent exhibits an altered immunological profile compared to an HBsAg from a reference HBV, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

Even more particularly, the present invention provides an isolated variant HBsAg or a recombinant or derivative form thereof or a chemical equivalent thereof wherein said HBsAg or its recombinant or derivative form or its chemical equivalent comprises an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to an HBsAg from a reference HBV and wherein a neutralizing antibody directed to a reference HBV exhibits no or reduced neutralising activity to an HBV carrying said variant HBsAg, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

Preferred mutations in the HBV DNA polymerase include variants selected from patients with HBV recurrence following ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV treatment. Nucleoside or nucleotide analog or other anti-HBV agents treatment may occur in relation to a transplantation procedure (e.g. bone marrow transplantation (BMT) or OLT) or following treatment of patients diagnosed with hepatitis. Following selection of variants, viral loads are obtainable at levels similar to pre-treatment levels or are increasing while on therapy.

Preferred mutations include, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236Tand rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment, rtH90D, and rtL/F108L; in even yet another embodiment, rtL157L/M, rtA181V and rtV207I; in still yet another embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and

rtN238N/H; in a further embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37; in yet another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63; in still another embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91; in even yet another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in still yet another embodiment, rtM204 and rtY203; in another embodiment, rt235, rt236, rt237, rt238 and rt239; in a further embodiment, rt247, rt248, rt249, rt250 and rt251; in yet another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;

V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;

A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;

Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;

P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;

Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;

F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y.

Reference above to "deletion" means that the first mentioned amino acid before the residue number has been deleted.

Such HBV variants are proposed to exhibit a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. It should be noted that the nomenclature system for amino acid positions is based on the methionine residues in the YMDD motif being designated codon rtM204. This

numbering system is different to that in Australian Patent No. 734831 where the methionine residue in the YMDD motif within the polymerase gene is designated codon 550. In this regard, rtL180M and rtM204V correspond to L526M and M550V, respectively, in Australian Patent No. 734831. Corresponding mutations may also occur in envelope genes such as in one or more of PreS1, PreS2 and S. The mutations in S gene encoding HBsAg at sT118R, sP120T, sS143S/T, sD144A or sI195M also result in mutation in the in the polymerase gene rtY126C, rtT128N, rtF151S/F or rtM204V respectively.

Another potential mode of action of ADV and other acyclic nucleoside phosphonates is that of immune stimulation (Calio *et al., Antiviral Res. 23:* 77-89, 1994). A number of mutations resulted in changes in the envelope gene detected in HBV variants which may be associated with immune escape. These changes include sT118R, sP120T, sS126T, sM133T, sM133L/M, sF134V, sS143S/T, sD144A, sG145A and/or sW172STOP.

HBV encoding the mutation at codon sG145R is a well characterized vaccine escape mutant, although the envelope protein from HBV encoding the mutation at sG145A does not have the same antigen/antibody binding characteristics as the sG145R. This mutation was detected in HBV isolated from patient C in conjunction with mutations at codons 143 and 144.

The identification of the variants of the present invention permits the generation of a range of assays to detect such variants. The detection of such variants may be important in identifying resistant variants to determine the appropriate form of chemotherapy and/or to monitor vaccination protocols, or develop new or modified vaccine preparations.

Still another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other

nucleoside or nucleotide analogs or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G, and A domains through to E or a region proximal thereto of said DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents.

Preferably, the assay detects one or more of the following mutations: in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment, rtH90D and rtL/F108L; in even yet another embodiment, sP120T, sM125T and sT127A; in still yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in another embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145Aand sW172Stop; in a further embodiment, sN40S, sC69STOP, sM75I, sL88P, sT118A, sW182Stop, sW196L, sY206H and sY225F; in yet another embodiment, s181M and sP214Q; in still another embodiment, sF83S, sL173F and sW199L; in yet another embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in still another embodiment, sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop, sS207R; in even still

another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37); in another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63); in a further embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91);in yet another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in still another embodiment, rtM204 and rtY203; in even yet another embodiment, rt235, rt236, rt237, rt238 and rt239and in even still another embodiment, rt247, rt248, rt249, rt250 and rt251 and in another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M//deletionF;

H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;

V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;

A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;

Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;

P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;

F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Accordingly, another aspect of the present invention produces a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase and/or a corresponding region of the S gene, wherein the presence of a

mutation selected from, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment, rtH90D and rtL/F108L; in even yet another embodiment, sP120T, sM125T and sT127A; in still yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in another embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145Aand sW172Stop; in a further embodiment, sN40S, sC69STOP, sM75I, sL88P, sT118A, sW182Stop, sW196L, sY206H and sY225F; in yet another embodiment, s181M and sP214Q; in still another embodiment, sF83S, sL173F and sW199L; in yet another embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in still another embodiment, sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop, sS207R; in even still another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37); in another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63); in a further embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91);in yet another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in still another embodiment, rtM204 and rtY203; in even yet another embodiment, rt235, rt236, rt237, rt238 and rt239and in even still another embodiment, rt247, rt248, rt249, rt250 and rt251; and in another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. --

A further aspect of the present invention produces a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase and/or a corresponding region of the S gene, wherein the presence of a mutation selected from, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment, rtH90D and rtL/F108L; in even yet another embodiment, sP120T, sM125T and sT127A; in still yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in another embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145Aand sW172Stop; in a further embodiment, sN40S, sC69STOP, sM75I, sL88P, sT118A, sW182Stop, sW196L, sY206H and sY225F; in yet another embodiment, s181M and sP214Q; in still another embodiment, sF83S, sL173F and sW199L; in yet another embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in still another embodiment, sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop, sS207R; in even still another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37); in another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63); in a further embodiment,

rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91);in yet another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in still another embodiment, rtM204 and rtY203; in even yet another embodiment, rt235, rt236, rt237, rt238 and rt239and in even still another embodiment, rt247, rt248, rt249, rt250 and rt251; and in another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The detection of HBV or its components in cells, cell lysates, cultured supernatant fluid and bodily fluid may be by any convenient means including any nucleic acid-based detection means, for example, by nucleic acid hybridization techniques or *via* one or more polymerase chain reactions (PCRs). The term "bodily fluid" includes any fluid derived from the blood, lymph, tissue or organ systems including serum, whole blood, biopsy and biopsy fluid, organ explants and organ suspension such as liver suspensions. The invention

further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR) and reverse transcription polymerase chain reaction (RT-PCR), amongst others. Other forms of detection include Northern blots, Southern blots, PCR sequencing, antibody procedures such as ELISA, Western blot and immunohistochemistry. A particularly useful assay includes the reagents and components required for immobilized oligonucleotide- or oligopeptide-mediated detection systems.

One particularly useful nucleic acid detection system is the reverse hybridization technique. In this technique, DNA from an HBV sample is amplified using a biotin or other ligand-labeled primer to generate a labeled amplificon. Oligonucleotides immobilized to a solid support such as a nitrocellulose film are then used to capture amplified DNA by hybridization. Specific nucleic acid fragments are identified *via* biotin or the ligand. Generally, the labeled primer is specific for a particular nucleotide variation to be detected. Amplification occurs only if the variation to be detected is present. There are many forms of the reverse hybridization assay and all are encompassed by the present invention.

Detecting HBV replication in cell culture is particularly useful.

This and other aspects of the present invention is particularly amenable to microarray analysis such as to identify oligonucleotides including sense and antisense molecules, RNAi or siRNA molecules or DNA or RNA-binding molecules which down-regulate genomic sequences or transcripts of HBV. Microarray analysis may also be used to identify particular mutations in the HBV genome such as within the HBV DNA polymerase-coding region or the HBsAg-coding region.

Another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV by:

generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in a plasmid vector and then transfecting said cells with said construct;

contacting the cells, before, during and/or after transfection, with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agents; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In a preferred embodiment, the plasmid vector may include genes encoding part or all of oher viral vectors such as baculovirus or adenovirus (Ren and Nassal, 2001, *supra*) and the method comprises:

generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in or fused to an amount of a baculovirus genome or adenovirus genome effective to infect cells and then infecting said cells with said construct;

contacting the cells, before, during and/or after infection, with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In an alternative embodiment, the method comprises:

generating a continuous cell line comprising an infectious copy of the genome of the HBV in a replication competent effective amount such that said infectious HBV genome is stably integrated into said continuous cell line such as but not limited to 2.2.15 or AD;

contacting the cells with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to the agent; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

The above-mentioned methods are particularly useful in identifying or developing agents against HBV variants such as those carrying mutations, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a

further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment rtH90D and rtL/F108L; in even yet another embodiment, rtL157L/M, rtA181V and rtV207I; in even still another embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H; in a further embodiment, sP120T, sM125T and sT127A; in yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in still another embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop; in even yet another embodiment, sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182STOP, sW196L, sY206H and sY225F; in even still another embodiment, s181M and sP214Q; in another embodiment, sF83S, sL173F and sW199L; in a further embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in yet another embodiment, sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R; in still another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37; in even yet another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63; in even still another embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91; in another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in a further embodiment, rtM204 and rtY203; in yet another embodiment, rt235, rt236, rt237, rt238 and rt239 in still another embodiment, rt247, rt248, rt249, rt250 and rt251; and in even yet another embodiment,K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion.

Accordingly, another aspect of the present invention contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other potential anti-HBV agent, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence of the envelope genes or DNA polymerase gene selected from, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment rtH90D and rtL/F108L; in even yet another embodiment, rtL157L/M, rtA181V and rtV207I; in even still another embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H; in a further embodiment, sP120T, sM125T and sT127A; in yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in still another embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop; in even yet another embodiment, sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182STOP, sW196L, sY206H and sY225F; in even still another embodiment, s181M and sP214Q; in another embodiment, sF83S, sL173F and sW199L; in a further embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in yet another embodiment, sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R; in still another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37; in even yet another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63; in even still another embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91; in another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in a further embodiment, rtM204 and rtY203; in yet another embodiment, rt235, rt236,

rt237, rt238 and rt239 in still another embodiment, rt247, rt248, rt249, rt250 and rt251; and in even yet another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/QE; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The detection of amino acid variants of DNA polymerase is conveniently accomplished by reference to the amino acid sequence shown in Formulae I and II. The polymorphisms shown represent the variations shown in various databases for active pathogenic HBV strains. Where an HBV variant comprises an amino acid different to what is represented, then such an isolate is considered a putative HBV variant having an altered DNA polymerase activity.

The present invention further contemplates agents which inhibit ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV,

FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV resistant HBV variants. Such agents are particularly useful if long term treatment by ADV, LMV, FTC and/or TFV and/or optionally other nucleoside analogs or nucleotide analogs such as TFV is contemplated by the clinician. The agents may be DNA or RNA or proteinaceous or non-proteinaceous chemical molecules. Natural product screening such as from plants, coral and microorganisms is also contemplated as a useful potential source of masking agents as is the screening of combinatorial or chemical libraries. The agents may be in isolated form or in the form of a pharmaceutical composition or formulation and may be administered in place of or sequentially or simultaneously with a nucleoside or nucleotide analog. Furthermore, rationale drug design is contemplated including solving the crystal or NMR structure of, for example, HBV DNA polymerase and designing agents whicih can bind to the enzyme's active site. This approach may also be adapted to other HBV components.

Accordingly, another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, , said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in a plasmid vector and then transfecting said cells with said construct;

contacting said cells, before, during and/or after transfection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

Still another aspect of the present invention provides a method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, , said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct;

contacting said cells, before, during and/or after infection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

Preferably, the HBV genome is stably integrated into the cells' genome.

Particularly useful cells are 2.2.15 cells (Price *et al., Proc. Natl. Acad. Sci. USA 86(21):* 8541-8544, 1989 or AD cells (also known as HepAD32 cells or HepAD79 cells [Ying *et al., Viral Hepat. 7(2):* 161-165, 2000.

Whilst the baculovirus vector is a particularly useful in the practice of the present invention, the subject invention extends to a range of other vectors such as but not limited to adenoviral vectors.

The present invention further extends to cell lines (e.g. 2.2.15 or AD cells) carrying genetic constructs comprising all or a portion of an HBV genome or a gene or part of a gene therefrom.

The present invention also provides for the use of the subject HBV variants to screen for anti-viral agents. These anti-viral agents inhibit the virus. The term "inhibit" includes antagonizing or otherwise preventing infection, replication, assembly and/or release or any intermediate step. Preferred anti-viral agents include nucleoside or nucleotide analogs or anti-HBV agents, however, the present invention extends to non-nucleoside molecules.

In addition, rational drug design is also contemplated to identify or generate chemical molecules which either mimic a nucleoside or which interact with a particular nucleotide sequence or a particular nucleotide. Combinatorial chemistry and two hybrid screening are some of a number of techniques which can be employed to identify potential therapeutic or diagnostic agents.

In one example, the crystal structure or the NMR structure of polymerase or the surface antigen is used to rationally design small chemical molecules likely to interact with key regions of the molecule required for function and/or antigenicity. Such agents may be useful as inhibitors of polymerase activity and/or may alter an epitope on the surface antigen.

Several models of the HBV polymerase have been prepared due to the similarity with reverse transcriptase from HIV (Das *et al., J. Virol. 75(10):* 4771-4779, 2001; Bartholomeusz *et al., Intervirology 40(5-6):* 337-342 1997; Allen *et al., Hepatology 27(6):* 1670-1677, 1998). The models of the HBV polymerase can be used for the rational drug design of new agents effective against HBV encoding the resistant mutations as well as wild type virus. The rational drug that is designed may be based on a modification of an existing antiviral agent such as the agent used in the selection of the HBV encoding the mutations associated with resistance. Viruses or clones expressing HBV genomic material encoding the mutations may also be used to screen for new antiviral agents.

In an alternative embodiment, the present invention also contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV polymerase in an *in vitro* polymerase assay. The HBV polymerase activity can be examined using established assays (Gaillard *et al., Antimicrob Agents Chemother. 46(4):* 1005-1013, 2002; Xiong *et al., Hepatology 28(6):* 1669-1673, 1998).

As indicated above, microarray technology is also a useful means of identifying agents which are capable of interacting with defined HBV internal or external components. For example, arrays of HBV DNA polymerase or peptide fragments thereof carrying different amino acid variants may be used to screen for agents which are capable of binding or otherwise interacting with these molecules. This is a convenient way of determining the differential binding patterns of agents between HBV variants. Arrays of antibodies may also be used to screen for altered HBsAg molecules. Microarrays are also useful in proteomic analysis to identify molecules such as antibodies, interferons or cytokines which have an ability to interact with an HBV component. Microarrays of DNA and RNA molecules may also be employed to identify sense and antisense molecules for genetic regions on the HBV genome or transcripts thereof.

The above methods are particularly useful in identifying an inhibitor of an HBV resistant to or exhibiting reduced sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV

and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. The present invention extends, therefore, to compositions of the inhibitors. The inhibitors may also be in the form of antibodies or genetic molecules such as ribozymes, antisense molecules and/or sense molecules for co-suppression or the induction of RNAi or may be other nucleoside or nucleotide analogs or other anti-HBV agents or derivatives of known analogs. Reference to RNAi includes reference to short, interfering RNAs (siRNA).

The term "composition" includes a "pharmaceutical composition" or a formulation.

The inhibitor is referred to below as an "active ingredient" or "active compound" and may be selected from the list of inhibitors given above.

The composition may include an antigenic component of the HBV, a defective HBV variant or an agent identified through natural product screening or rational drug design (including combinatorial chemistry).

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of encoding an aspartyl protease inhibitor. The vector may, for example, be a viral vector.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dilution medium comprising, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of superfactants. The preventions of the action of microorganisms can be brought about by various anti-bacterial and anti-fungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with the active ingredient and optionally other active ingredients as required, followed by filtered sterilization or other appropriate means of sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, suitable methods of preparation include vacuum drying and the freeze-drying technique which yield a powder of active ingredient plus any additionally desired ingredient.

When the active ingredient is suitably protected, it may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active ingredient may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight

of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 200 mg of active compound. Alternative dosage amounts include from about 1 μg to about 1000 mg and from about 10 μg to about 500 mg. These dosages may be per individual or per kg body weight. Administration may be per hour, day, week, month or year.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavouring. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

As stated above, the present invention further extends to an isolated HBsAg from the HBV variants herein described. More particularly, the present invention provides an HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof. The isolated surface component and, more particularly, isolated surface antigen or its recombinant, derivative or chemical equivalents are useful in the development of biological compositions such as vaccine formulations.

Yet another aspect of the present invention provides a composition comprising a variant HBV resistant to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or an HBV surface antigen from said variant HBV or a recombinant or derivative form thereof or its chemical equivalent and one or more pharmaceutically acceptable carriers and/or diluents. Such a composition may be regarded as a therapeutic composition and is useful in generating an immune response including a humoral response. Generally, the HBV variants are "defective" and in themselves are unable to cause a sustained infection in a subject.

As indicated above, antibodies may be generated to the mutant HBV agents and used for passive or direct vaccination against infection by these viruses. The antibodies may be generated in humans or non-human animals. In the case of the latter, the non-human antibodies may need to be deimmunized or more specifically humanized prior to use. Deimmunized may include, for example, grafting complimentarity determining regions (CDRs) from the variable region of a murine or non-human animal anti-HBV antibody onto a human consensus fragment antibody binding (Fab) polypeptide. Alternatively, amino acids defining epitopes in the variable region of the antibody may be mutated so that the epitopes are no longer recognized by the human MHC II complex.

Insofar as ribozyme, antisense or co-suppression (RNAi) or siRNA or complexes thereof repression is concerned, this is conveniently aimed at post-transcription gene silencing. DNA or RNA may be administered or a complex comprising RNAi or a chemical analog thereof specific for HBV mRNA may be employed.

All such molecules may be incorporated into pharmaceutical compositions.

In another embodiment, the present invention provides a biological composition comprising a variant HBV or an HBsAg or L, M or S proteins from said variant HBV or a recombinant or derivative form thereof or its chemical equivalent.

Generally, if an HBV is used, it is first attenuated. The biological composition according to this aspect of the present invention generally further comprises one or more pharmaceutically acceptable carriers and/or diluents.

The biological composition may comprise HBsAg or like molecule from one HBV variant or the composition may be a cocktail of HbsAgs or L, M or S proteins or like molecules from a range of ADV- and/or LMV- and/or, FTC- and/or TFV-resistant HBV variants. Similar inclusions apply where the composition comprises an HBV.

The present invention is further directed to the use of defective HBV variants in the manufacture of therapeutic vaccines to vaccinate individuals against infection by HBV strains having a particular nucleotide sequence or encoding a particular polymerase or surface antigen or L, M or S proteins.

Examples of suitable vaccine candidates are defective forms of HBV variants comprising a mutation selected from, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment rtH90D and rtL/F108L; in even yet another embodiment, rtL157L/M, rtA181V and rtV207I; in even still another embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H; in a further embodiment, sP120T, sM125T and sT127A; in yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in still another embodiment, sS126T,

sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop; in even yet another embodiment, sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182STOP, sW196L, sY206H and sY225F; in even still another embodiment, s181M and sP214Q; in another embodiment, sF83S, sL173F and sW199L; in a further embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in yet another embodiment, sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R; in still another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37; in even yet another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63; in even still another embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91; in another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in a further embodiment, rtM204 and rtY203; in yet another embodiment, rt235, rt236, rt237, rt238 and rt239 in still another embodiment, rt247, rt248, rt249, rt250 and rt251; and in even yet another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;

V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;

A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;

P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or a combination of two or more mutations.

In one embodiment, for example, an HBV variant may be identified having a particular mutation in its polymerase conferring resistance or decreased sensitivity to a nucleoside analog. This variant may then be mutated to render it defective, i.e. attenuated or unable to

cause infection. Such a defective, nucleoside analog-resistant virus may then be used as a therapeutic vaccine against virulent viruses having the same mutation in its polymerase.

The subject invention extends to kits for assays for variant HBV resistant to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV.. Such kits may, for example, contain the reagents from PCR or other nucleic acid hybridization technology or reagents for immunologically based detection techniques. A particularly useful assay includes the reagents and components required for immobilized oligonucleotide- or oligopeptide-mediated detection systems.

Still another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G, and domains A through to E or a region proximal thereto of said DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV.

An assessment of a potential viral variant is important for selection of an appropriate therapeutic protocol. Such an assessment is suitably facilitated with the assistance of a computer programmed with software, which *inter alia* adds index values (I_Vs) for at least two features associated with the viral variants to provide a potency value (P_A) corresponding to the resistance or sensitivity of a viral variant to a particular chemical compound or immunological agent. The I_Vs can be selected from (a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent; (b) an altered DNA polymerase from wild-type HBV; (c) an altered surface antigen from wild-type HBV; or (d) morbidity or recovery potential of a patient. Thus, in accordance with the present invention, I_Vs for such features are stored in a machine-readable storage medium, which is capable of processing the data to provide a P_A for a particular viral variant or a biological specimen comprising same.

Thus, in another aspect, the invention contemplates a computer program product for assessing the likely usefulness of a viral variant or biological sample comprising same for determining an appropriate therapeutic protocol in a subject, said product comprising:

(1) code that receives as input I_Vs for at least two features associated with said viral agents or biological sample comprising same, wherein said features are selected from:

(a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
(b) an altered DNA polymerase from wild-type HBV;
(c) an altered surface antigen from wild-type HBV;
(d) morbidity or recovery potential of a patient; or
(e) altered replication capacity (increased or decreased);

(2) code that adds said I_Vs to provide a sum corresponding to a P_V for said viral variants or biological samples; and

(3) a computer readable medium that stores the codes.

In a related aspect, the invention extends to a computer for assessing the likely usefulness of a viral variant or biological sample comprising same in a subject, wherein said computer comprises:

(1) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise I_Vs for at least two features associated with said viral variant or biological sample; wherein said features are selected from:-

(a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
(b) an altered DNA polymerase from wild-type HBV;
(c) an altered surface antigen from wild-type HBV;
(d) morbidity or recovery potential of a patient; or
(e) altered replication capacity (increased or decreased);

(2) a working memory for storing instructions for processing said machine-readable data;

(3) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine readable data to provide a sum of said I_Vs corresponding to a P_V for said compound(s); and

(4) an output hardware coupled to said central processing unit, for receiving said P_V.

Any general or special purpose computer system is contemplated by the present invention and includes a processor in electrical communication with both a memory and at least one input/output device, such as a terminal. Figure 19 shows a generally suitable computer system. Such a system may include, but is not limited, to personal computers, workstations

or mainframes. The processor may be a general purpose processor or microprocessor or a specialized processor executing programs located in RAM memory. The programs may be placed in RAM from a storage device, such as a disk or pre-programmed ROM memory. The RAM memory in one embodiment is used both for data storage and program execution. The computer system also embraces systems where the processor and memory reside in different physical entities but which are in electrical communication by means of a network.

In an alternative embodiment, the program screens for a mutation selected from, in one embodiment, rtS21A, rtL122F, rtN124H, rtH126R, rtT28N, rtP130Q, rtD131N and rtY135C; in another embodiment, rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M; in a further embodiment, rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D; in yet another embodiment, rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A; in still another embodiment rtH90D and rtL/F108L; in even yet another embodiment, rtL157L/M, rtA181V and rtV207I; in even still another embodiment, rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K; in another embodiment, rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H; in a further embodiment, sP120T, sM125T and sT127A; in yet another embodiment, sT118R, sM133T, SF134V, sI195M, sS207R and sY225Y/C; in still another embodiment, sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop; in even yet another embodiment, sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182STOP, sW196L, sY206H and sY225F; in even still another embodiment, s181M and sP214Q; in another embodiment, sF83S, sL173F and sW199L; in a further embodiment, sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C; in yet another embodiment, sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R; in still another embodiment, rtK32, rtN33, rtP34, rtH35 and rtT37; in even yet another embodiment, rtP59, rtK60, rtF61, rtA62 and rtV63; in even still another embodiment, rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91; in another embodiment, rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184; in a further embodiment, rtM204 and rtY203; in yet another embodiment, rt235, rt236,

rt237, rt238 and rt239 in still another embodiment, rt247, rt248, rt249, rt250 and rt251; and in even yet another embodiment,

K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;

F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;

V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;

A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;

P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;

Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;

F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;

Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;

L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;

N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or a combination of two or more mutations.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Overlapping genome of HBV

The overlapping genome of HBV is represented in Figure 1. The gene encoding DNA polymerase (P), overlaps the viral envelope genes, Pre-S1 and Pre-S2, and partially overlaps the X and core (C) genes. The HBV envelope comprises small, middle and large proteins HBV surface antigens. The large protein component is referred to as the HBV surface antigen (HBsAg) and is encoded by the S gene sequence. The Pre-S1 and Pre-S2 gene sequences encode the other envelope components.

EXAMPLE 2

Patients and Treatment

Patient A, a 48 year old Lebanese woman was initially referred for evaluation of thrombocytopenia and hepatosplenomegaly. At this time the patient had abnormal LFT's (ALT 67 U/L, normal <55) and the HBV DNA was 61 pg/ml (231 days prior to the start of treatment). The patient was HBsAg and HBeAg positive. The ALT's fluctuated between 50-70 IU/L from (-231 to -35 days pretreatment). ADV was commenced on Day 0 in a clinical trial on 30 mg/day. HBV DNA levels were reduced with ADV treatment. The ADV treatment was reduced to 10 mg /day (144 days post-treatment). There was a problem with the randomization treatment protocol. The patient was on antiviral treatment for 1 month only during the second year of the treatment period. The study was completed on Day 679 post ADV treatment. The patient was not on ADV treatment until the open label ADV was recommenced on Day 875 from the start of the initial ADV treatment. This second period of ADV treatment was given for 108 days (day 983 post initial ADV treatment). The HBV DNA levels remained at 7-10 pg/ml (1.96×10^5 to 2.8×10^5 copies/ml). At Day 983, ADV treatment was stopped and the patient was treated with LMV.

Patient B is a male liver transplant patient. The patient has been on both sequential and combination antiviral therapy including HBIG, FCV+HBIG, LMV+HBIG, LMV,

LMV+GCV, LMV+FCV+GCV, LMV+GCV and finally LMV+ADV. The patient has been on long term ADV+LMV treatment for over 795 days.

Patient C, is a 58 year old male. Prior to ADV treatment the patient had abnormal LFT's (ALT 240 IU/L, normal <55) and the HBV DNA was $2x10^7$copies/ml. ADV was commenced on Day 0 in a clinical trial on 10 mg/day for two years. The average ALT during the two year clinical trial period ws 114 IU/L. However, the ALT was rising and at 630 days after the start of ADV treatment the ALT remained high 407 IU/L. Open label ADV was commenced on Day 668 from the start of the initial ADV treatment. This second period of ADV treatment was given for 71 days. The HBV DNA levels remained high during open label ADV treatment ($3.7x10^6$ to $1.5x10^7$ copies/ml). The peak ALT during open label ADV treatment was 517 IU/L (Day 738). The next day (Day 739), ADV treatment was stopped and the patient was treated with LMV.

EXAMPLE 3

Detection of Viral Markers

Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), anti-HBe and hepatitis B core antigen (HBcAg) specific IgG and IgM were measured using commercially available immunoassays (Abbott Laboratories, North Chicago, IL, USA). Hepatitis B viral DNA levels were measured using a capture hybridization assay according to the manufacturer's directions (Digene Hybrid Capture II, Digene Diagnostics Inc., Beltsville, MD). The manufacturers stated cut-off for detecting HBV viremia in clinical specimens was $0.7x10^6$ copies/ml or 2.5 pg/ml, [Hendricks *et al., Am J Clin Pathol 104:* 537-46, 1995]. HBV DNA levels can also be quantitated using other commercial kits such as Cobas amplification HBV monitor kit (Roche).

EXAMPLE 4

Sequencing of HBV DNA

HBV DNA was extracted from 100 µl of serum as described previously by Aye *et al.*, *J. Hepatol. 26:* 1148-1153, 1997. Oligonucleotides were synthesized by Geneworks, Adelaide, Australia. Amplification of the HBV polymerase gene has been described by Aye *et al.*, 1997, *supra.*

The specific amplified products were purified using PCR purification columns from MO BIO Laboratories Inc (La Jolla, CA) and directly sequenced using Big Dye terminator Cycle sequencing Ready Reaction Kit (Perkin Elmer, Cetus Norwalk, CT). The PCR primers were used as sequencing primers, **OS1** 5'- GCC TCA TTT TGT GGG TCA CCA TA-3' (nt 1408-1430) [SEQ ID NO:3], **TTA3** 5'-AAA TTC GCA GTC CCC AAA-3'(nt2128-2145) [SEQ ID NO:4], **JM** 5'-TTG GGG TGG AGC CCT CAG GCT -3'(nt1676-1696) [SEQ ID NO:5], **TTA4** 5'-GAA AAT TGG TAA CAG CGG -3' (nt 2615-2632) [SEQ ID NO:6], OS2 5' TCT CTG ACA TAC TTT CCA AT 3' (nt 2798-2817) [SEQ ID NO:7], to sequence the internal regions of the PCR products.

EXAMPLE 5

Analysis of HBV DNA

Patient A: During ADV treatment, unique HBV mutations were detected by sequencing (Tables 4 and 5) This includes the unique mutation at rtY135C in addition to the mutation at rtT128N that was present prior to ADV treatment. A number of other unique changes were also detected in the polymerase and in the overlapping envelope gene (Table 5, Figures 4, 5 and 6). The unique change in the HBsAg include sP120T. These unique changes were compared to reference sequences from each of the seven genotypes A-G as well as a consensus sequence from pretreatment samples to determine unique changes.

Patient B: The HBV mutations prior to ADV treatment and during ADV treatment are listed in Table 6 and 7 and Figures 7, 8, and 9. The unique changes in the rt region of the HBV DNA polymerase include rtN/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M. The unique changes in the HBsAg include sT118R, sM133T, sF134V, sI195M, sS207R, sY225Y/C.

Patient C: The HBV mutations prior to ADV treatment and during ADV treatment are listed in Tables 8 and 9 and Figures 10, 11 and 12. The unique changes in the rt region of the HBV DNA polymerase include rtN53D, rtS116P, rtF151F/T, rtN236T and rtN238D. The unique changes in the HBsAg include sG145A and sW172stop.

Patient D: The HBV mutations during ADV treatment is listed in Table 10 and Figures 13, 14 and 15. The unique changes in the HBV DNA polymerase include rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A. The unique changes in the surface include sN40S and sC69 Stop. A number of unique changes were detected after the stop codon mutation at codon 69 of the S gene including sM75I, sL88P, sT118A, sW182stop, sW196L, sY206H and sY225F.

Patient E: The HBV mutations during ADV treatment is listed in Table 11 and Figures 16, 17 and 18. The unique changes in the HBV DNA polymerase include rtH90D and rtL/F108L. The unique changes in the surface include sI81M and sP214Q. A six nucleotide insertion was also detectedresulting in a two amino acid insertion in the HBV polymerase and envelope gene at codons rt131 and s122, respectively. This insertion was previously detected in pre-ADV samples.

EXAMPLE 6

Adefovir Dipivoxil (ADV)

ADV (formerly Bis-pom PMEA)) is a potent inhibitor of HBV replication. The structure of ADV is shown in Figure 2 and its synthesis is described by Benzaria *et al., J Med Chem. 39:* 4958-4965, 1996).

EXAMPLE 7

HBV rt mutants

The HBV polymerase has similarities to other polymerases including HIV. Thus, mutations associated with resistance to antiviral agents may occur within the polymerase in functionally important regions such as the nucleotide triphosphate binding pocket that may also include the interaction between the DNA primer and template strand, magnesium ions and nucleoside triphosphates or nucleoside/ nucleotide analogs (and there vaiorus phosphroylated forms). Codons which are proposed to be mutated during anti-viral selection pressure are rtK32, rt N33, rtP34, rtH35 and rtT37 (that are upstream from the F domain); rt P59, rtK60, rtF61, rtA62 and rtV63 (between the F and A domains), rtD83, rtV84, rtS85, rtA86, rt Y89, rt H90 and rtI/L91 (within the A domain and the region immediately prior to and after), rtP177, rtF178, rt L179, rtL180, rtA181, rtQ182, rtF183 and rtT184 (B domain); rtM204 and rtY203(C Domain), rtL235, rtN236, rtP/T237, rtN/H/A/S/Q238 and rtK239 (D Domain), rLt247, rtN/H248, rtF249, rtM250 and rtG251 (E Domain). The codons are defined in Table 12 and examples of various mutants are given in Tables 13 and 14.

EXAMPLE 8

Patient F

The HBV mutations during ADV treatment of Patient F are listed in Table 15 and Figures 20, 21 and 22. The unique changes in the HBV DNA polymerase includes rtL157L/M, rtA181V, rtV207I, and rtN236T. The unique changes in the surface includes sF83S, sL173F and sW199L.

EXAMPLE 9

Patient G

The HBV mutations during ADV treatment of Patient G are listed in Table 16 and Figures 23, 24 and 25. The unique changes in the HBV DNA polymerase includes rtL80V, rtP109S, rtI163V, rtM204I, rtL229M and rtN/H/A/S/Q238K. The unique changes in the surface includes sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C.

EXAMPLE 10

Patient H

The HBV mutations during ADV treatment in Patient H are listed in Table 17 and Figures 26, 27 and 28. The unique changes in the HBV DNA polymerase includes rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E, and rtN238N/H. The unique changes in the surface include sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R.

EXAMPLE 11

In vitro analysis of ADV resistance

The sensitivity/resistance profile of HBV mutants to ADV was examined *in vitro* using recombinant HBV/baculovirus. The procedure for analyzing the resistance profile is outlined in the following Examples 12-20.

EXAMPLE 12

Cell culture

Sf21 insect cells were maintained in supplemented Grace's insect medium further supplemented with 10% v/v heat-inactivated fetal bovine serum (Gibco BRL,

Gaithersburg, MD) in humidified incubator at 28°C with CO_2. HepG2 cells were maintained in minimal essential medium supplemented with 10% v/v heat-inactivated fetal bovine serum (MEM-FBS). HepG2 cells were grown in humidified 37°C-incubators at 5% v/v CO_2.

EXAMPLE 13

Preparation of HBV/baculovirus transfer vector with specific point mutations

The recombinant HBV/baculovirus system used for antiviral testing has been previously described (Delaney *et al., Antimicrob Agents Chemother 45(6):* 1705-1013, 2001). In brief, the recombinant transfer vector was created by excising a fragment containing the 1.3x HBV genome construct and cloning it into the multiple cloning region of a baculovirus vector pBlueBac4.5 (Invitrogen, Carlsbad, CA). Point mutations were created by site directed mutagenesis using the commercial kits according to the manufacturer's specifications (QuikChange, Stratagene). HBV/ baculovirus recombinant clones encoding the reverse transcriptase mutations rtA181T/N236T/N238D and rtN236T/N236D in combination with the precore mutation at G1896A (pcW28 stop) or wild-type with respect to codon pcW28, were prepared by site-directed mutagenesis. The nucleotide sequence of the plasmid and the point mutations generated by site directed mutagenesis were confirmed by sequencing using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer's specifications (Perkin Elmer, Cetus Norwalk, CT).

EXAMPLE 14

Generation of recombinant baculoviruses containing the 1.3 HBV construct

Purified recombinant transfer vector and linear AcMNPV baculovirus DNA were co-transfected into Sf21 cells using the BacNBlue transfection kit from Invitrogen (Carlsbad, CA); recombinant viruses were isolated by plaque assay according to the manufacturer's instructions. A series of recombinant viruses were amplified from isolated plaques by infecting 100-mm dishes of Sf21 cells. Viral DNA was extracted from amplified viruses

using standard procedures. Purified viral DNA was digested with restriction enzymes and then fractionated by electrophoresis in a 1% v/v agarose gel. Southern blotting was performed to determine which virus isolates contained the intact 1.3 HBV construct. A Boehringer Mannheim Random Prime DNA Labeling kit (Indianapolis, IN) was used to generate [P^{32}]-radiolabeled probes. A full-length double-stranded HBV genome was used as a template for all radiolabeled probes. Viral DNA sequence was confirmed by PCR amplification of the polymerase catalytic region using the sense primer 5'-GCC TCA TTT TGT GGG TCA CCA TA-3' [SEQ ID NO:8], (nucleotide 1408 to 1430 according to HBV Genebank Accession number M38454) and the antisense primer 5'-TCT CTG ACA TAC TTT CCA AT-3' [SEQ ID NO:9] (nucleotides 2817 to 2798 according to HBV Genebank Accession number M38454). The following primers were utilized for the sequencing of internal regions 5'-TGC ACG ATT CCT GCT CAA-3' [SEQ ID NO:10] (nucleotides 2345-2362 according to HBV Genebank Accession number M38454) and 5'-TTT CTC AAA GGT GGA GAC AG-3' [SEQ ID NO:11] (nucleotides 1790-1810 according to HBV Genebank Accession number M38454).

EXAMPLE 15

Preparative baculovirus amplification and purification

Baculoviruses were amplified by infecting suspension cultures of Sf21 cells in log phase at a multiplicity of infection (moi) of 0.5 pfu/cell. Infections were allowed to proceed until a majority of the cells in the flasks showed visible signs of infection (four to five days). Virions were concentrated from infected Sf21 medium by centrifugation at 80,000 x g and purified through a 20-60% w/v sucrose gradient. Purified virus was titrated in quadruplicate in Sf21 cells by end-point dilution. An aliquot of each high titer stock was used for DNA extraction. The polymerase gene was amplified and sequenced to confirm the presence of the site-directed mutagenesis as in Example 14.

EXAMPLE 16

Infection of HepG2 cells with recombinant HBV expressing baculovirus

HepG2 cells were seeded at approximately 20-40% confluency and then were grown for 16-24 hours before infection. On the day of infection, triplicate plates of cells were trypsinized, and viable cell number was determined with a hemocytometer using Trypan blue exclusion. Average cell counts were calculated and used to determine the volume of high-titer viral stock necessary to infect cells at the indicated moi. HepG2 cells were washed one time with serum-free MEM to remove traces of serum. Baculovirus was diluted into MEM without serum to achieve the appropriate moi using volumes of 1.0, 0.5, and 0.25 ml to infect 100-mm, 60 mm, and 35-mm dishes, respectively. Baculovirus was adsorbed to HepG2 cells for one hour at 37°C with gentle rocking every 15 minutes to ensure that the inoculum was evenly distributed. The inoculum was then aspirated and HepG2 cells were washed two times with phosphate-buffered saline and refed MEM-FBS with or without various concentrations of agents.

EXAMPLE 17

Detection of intracellular replicative intermediates

HBV core particles were isolated from the cytoplasmic fraction of HepG2 cells lysed in 0.5% w/v NP-40. Cytoplasmic extracts were adjusted to 10 mmol/l McC12 and unprotected DNA was removed by an incubation to 500 g/ml Proteinase K for 1.5 hours at 37°C. HBV DNA in the samples were then extracted using commercial DNA extraction kits such as Qiagen (DNA extraction) or in-house methods using sequential phenol and chloroform extractions, and the nucleic acids were recovered by ethanol precipitation. Nucleic acids were resuspended in 50 µl /l TE (10 mmol/l Tris, 1 mmol/l ethylenediaminetetraacetic acid), normalized by OD260, and digested with 100 g/ml RNase (Boehringer Mannheim, Indianapolis, IN) for one hour at 37°C before analysis by real-time PCR or electrophoresis and Southern blotting. After southern blot analysis a BioRad GS-670 imaging densitometer and the Molecular Analyst software (BioRad, Hecules California) was used to analyze suitable exposures of Southern blots.

Densitometry data was fitted to logistic dose response curves using the TableCurve 2D software package from Jandel Scientific. Logistic dose response equations were used to calculate IC_{50} and IC_{90} values and co-efficients of variation.

EXAMPLE 18

Real-time PCR

For the real-time PCR based assay for HBV, HBV DNA was extracted from 200 µl of serum using the QIAamp DNA Mini Kit according to the manufacturer's instructions (QIAGEN GmbH, Hildens, Germany). Primers and a molecular beacon were designed for conserved nucleic acid sequences within the precore domain of the HBV genome to amplify and detect a 216-nucleotide product. Amplification was performed in a 50-µl reaction mixture containing 1.0 Taqman buffer A (Applied Biosystems, Foster City, CA), 3.0 mM MgCl, 0.4 pmol of each primer per µL, forward primer, PC1 (5'-GGGAGGAGATTAGGTTAA-3' [SEQ ID NO:12]) and reverse primer, PC2 (5'-GGCAAAAACGAGAGTAACTC-3' [SEQ ID NO:13]), 0.4 pmol of the HBV-specific molecular beacon per µL, (5'-FAM-CGCGTCCTACTGTTCAAGCCTCCAAGCTGT GACGCG-DABCYL-3' [SEQ ID NO:14]; where FAM represents fluorophore 6-carboxyfluorescein and DABCYL, 4-dimethylaminophenylazobenzoic acid, a quenching chromophore) and 1.25U of AmpliTaq Gold DNA polymerase (Perkin-Elmer). PCR was performed using the ABI PRISM 7700 spectrofluorometric thermocycler (Applied Biosystems). The PCR program consisted of an initial cycle (95°C for 10 minutes) followed by 45 amplification cycles (94°C for 15 secs, 50°C for 30 secs, 72°C for 30 secs). The instrument detected and recorded the fluorescence spectrum of each reaction tube during the annealing phase.

An external standard was constructed by ligation of a 1.3 kB wild-type HBV plasmid (genotype D) into the pBlueBac plasmid vector (Hershey Medical Center, Hershey, PA). Quantification of the DNA concentration of the plasmid was determined by spectrophotometry. Duplicates of serial 10-fold dilutions of the plasmid ranging from 10_8 copies/ml to 100 copies/ml were included in each run in order to generate a standard curve.

The copy number in each experimental reaction was determined by interpolation of the derived threshold cycle (C_T).

EXAMPLE 19

ADV treatments

ADV was resuspended in sterile water, aliquoted, and frozen at -20°C to avoid repeated freezing and thawing of the drug. Medium containing ADV was prepared daily as needed using fresh aliquots of 3TC. In experiments in which ADV treatment was initiated after viral infection, HepG2 cells were exposed to the indicated concentration of ADV immediately after infection with HBV baculovirus. In experiments utilizing pretreatment with ADV, cells were fed medium containing ADV 16 hours prior to HBV baculovirus infection, HBV baculovirus infection was also carried out in medium containing ADV, and cells were refed fresh medium containing ADV immediately after completion of the infection and washing procedures.

EXAMPLE 20

Antiviral testing performed with wild-type and HBV/baculovirus encoding rtA181T/N236T/N238D and rtN236T/N236D

The *in vitro* antiviral drug cross-resistance testing of the HBV mutants is shown in Table 18. The laboratory reference strain of HBV (genotype D subtype ayw) containing the introduced D domain mutations demonstrated increased IC_{50} values against ADV (Table 18). The rt N236T/N238D mutation was associated with a twenty-three fold increase in IC_{50} against ADV. This was reduced to a five-fold increase when the rtA181T was also present and this triple HBV polymerase mutant was resistant to LMV.

TABLE 4 Clinical, virological and HBV sequencing data summary for Patient A while on open label ADV.

Days post-ADV treatment	HBV DNA copies/ml (pg/ml)	ALT IU/L	Treatment protocol	Key polymerase mutations detected by sequencing[1]
-230	$1.7\ 10^6$ (61)	67 U/L	pre-therapy	rtT/N128T/N rtQ/H/R215Q/stop
875			ADV recommenced	
904	1.55×10^6			
932	2.97×10^6			
959	1.76×10^6			
983	1.64×10^6	65	end ADV	rtT128N rtY135C

[1] Nomenclature according to Stuyver *et al.*, 2001, *supra*

TABLE 5 Summary of HBV mutations in patient A treated with ADV

Sample name	Days post-ADV treatment	Genotype	Polymerase*	Surface
ILA1	-230	D	rtA/S21A/S **rtT/N128T/N**** **rtQ/H/R215Q/stop**	**sP120P/T** **sI208I/L**
ILA2	904	D	rtA/S21S rtF122L rtR126H rtT/N128T/N rtQ130P rtN131D rtQstop/215Q rtH248N	sP/T120P sT125M **sI/I208I/L**
ILA3	932	D	rtA/S21S rtF122L rtR126H rtT/N128T/N rtQ130P rtN131D rtQstop/215Q rtH248N	sP/T120P sT125M sI/I208I/L
ILA4	983	D	rtS21A rtL122F rtN124H rtH126R **rtT128N** rtP130Q rtD131N **rtY135C**	**sP120T** sM125T sT127A

* Nomenclature according to Stuyver *et al.*, 2001, *supra.*

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 6 Clinical, virological and HBV sequencing data summary for Patient B while on open label ADV.

Days post-ADV treatment	HBV DN copies/ml (pg/ml)	ALT IU/L	Treatment protocol	Key polymerase mutations detected by sequencing[1]
-867(S0)	183	298	pre-therapy	**rtN/S/T/I/V53D** **rtV153G** **rtQ/E215S** **rtN248H**
-8(S6)	955	427	pre-ADV on LMV	rtI/L80L **rtY126Q** rtL180M rtS202G **rtI204V**
76(S8)	not detected	150	on ADV (20 mg) and LMV	rtN/S/T/I/V53D **rtY126Q** rtL180M **rtS202G** **rtI204V**
637(S12)	not detected	36	on ADV (5 mg) and LMV	rtN/S/T/I/V53D **rtY126Q** rtL180M **rtS202G** **rtI204V**
872(S15)	not detected	67	on ADV (5 mg) and LMV	**rtN/S/T/I/V53D** **rtY126Q** **rtL180M** **rtS202G** **rtI204V** **rtI235I/M**

[1] Nomenclature according to Stuyver *et al.*, 2001, *supra*

TABLE 7 Summary of HBV mutations in Patient B treated with ADV

Sample name	Days post-ADV treatment	Genotype	Polymerase*	Surface
S0	-867	D	**rtN/S/T/I/V53D** **rtV153G** **rtQ/E215S** **rtN248H**	**sM/K/L133T** **sF134V** **sS207R** **sL21V/L**
S6	-8	D	rtI/L80L **rtY126Q** rtL180M **rtS202G** **rtI204V**	**sT118R** **sM133T** **sF134V** **sI195M** **sS207R**
S8	76	D	**rtN/S/T/I/V53D** **rtY126Q** **rtL180M** **rtS202G** **rtI204V**	**sT118R** **sM133T** **sF134V** **sI195M** **sS207R**
S12	637	D	**rtN/S/T/I/V53D** **rtY126Q** **rtL180M** **rtS202G** **I204V**	**sT118R** **sM133T** **sF134V** **sI195M** **sS207R**
S15	872	D	**rtN/S/T/I/V53D** **rtY126Q** **rtL180M** **rtS202G** **rtI204V** **rtI235I/M**	**sT118R** **sM133T** **sF134V** **sI195M** **sS207R** **sY225Y/C**

* Nomenclature according to Stuyver *et al.*, 2001, *supra*

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 8 Clinical, virological and HBV sequencing data summary for Patient C while on open label ADV.

Days post-ADV treatment	HBV DNA copies/ml (pg/ml)	ALT IU/L	Treatment protocol	Key polymerase mutations detected by sequencing[1]
-26	2×10^7		pre-therapy	**rtN53D rtS116P rtD/N/S134V rtN238D**
0		240	ADV commenced clinical trial	
29		160		
630		407		
668			Open label ADV	
701	1.5×10^7	226		
730	3.7×10^6	361		**rtN53D rtS116P rtF151S/T rtA181T rtN236T rtN238D**
738		517		
739			end ADV, start LMV	

[1] Nomenclature according to Stuyver *et al.*, 2001, *supra*

TABLE 9 Summary of HBV mutations in Patient C treated with ADV

Sample name	Days post-ADV treatment	Genotype	Polymerase*	Surface
DRJ1299	-26	D	**rtN53D****	T126S
			rtY54H	**S204G**
			rtS57P	
			rtL91I	L209V
			rtS116P	**S210R**
			rtF122L	
			rtY124H	
			rtD/N/S134V	
			rtK212R	
			rtL217R	
			rtS219A	
			rtN238D	
DRJ1	730	D	**rtN53D**	sS126T
			rtY54H	sM133L/M
			rtS57P	sS143S/T
			rtL91I	sD144A
			rtS116P	**sG145A**
			rtF122L	**sW172Stop**
			rtY124H	
			rtV134D	
			rtY141Y/F	
			rtL145M	
			rtF151T/F	
			rtA181T	
			rtK212R	
			rtL217R	
			rtS219A	
			rtN236T	
			rtN238D	

* Nomenclature according to Stuyver *et al.*, 2001, *supra.*

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 10 Summary of HBV mutations in Patient D treated with ADV

Sample Name	Genotype	Polymerase*	Surface
02575908	D	**rtS78T** **rtV84M** **rtY126C** **rtV191I** **rtM204I** **rtV214A**	**sN40S** **sC69stop** **sM75I** **sL88P** sT118A **sW182STOP** **sW196L** sY206H **sY225F**

* Nomenclature according to Stuyver *et al.*, 2001, *supra*.

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 11 Summary of HBV mutations in Patient E treated with ADV

Sample Name	Genotype	Polymerase*	Surface
8123/02	A	**rtH90D** **rtL/F108L** 6nt insertion/duplication after codon rt131(aaQ&N)	**sI81M** sY/S100Y 6nt insertion/ duplication after codon s122 (aaT & K) **sP214Q**

* Nomenclature according to Stuyver *et al.*, 2001, *supra*.

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 12 Codons where mutations occur following exposure to nucleoside or nucleotide analogs

Region/ Domain	Original amino acid in reverse transcriptase (rt) and codon position	Nucleotide				
prior to F	K32	AAG	AAA			
	N33	AAT				
	P34	CCT				
	H35	CAC				
	T37	ACC				
F TO A	P59	CCA				
	K60	AAA				
	F61	TTC				
	A62	GCA				
	V63	GTC				
A	D83	GAT				
	V84	GTG				
	S85	TCT				
	A86	GCG				
	Y89	TAT				
	H90	CAT				
	I/L91	ATT	CTT			
B	P177	CCG				
	F178	TTT				
	L179	CTC				
	L180	CTG				
	A181	TTG				
	Q182	CAG				
	F183	TTT				
	T184	ACT				
C	Y203	TAT				
	M204	ATG				
D	L235	TTG	TTA			
	N236	AAC	AAT			
	T237	ACT	ACC			
	P237	CCT	CCC			
	N238	AAT	AAC			
	H238	CAC				
	A238	GCT				
	S238	TCT				
	Q238	CAG				

Region/ Domain	Original amino acid in reverse transcriptase (rt) and codon position	Nucleotide				
	K239	AAA	AAG			
E	L247	CTT	TTA	CTA	CTC	CTG
	N248	AAC	AAT			
	H248	CAT	CAC			
	F249	TTC	TTT			
	M250	ATG				
	G251	GGT	GGA	GGC	GGG	
	V251	GTC				

TABLE 13 Target amino acid sites in rt with codons and mutations leading to amino acid changes. I → II → III → IV

Title	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
K32	AAG	Lys	AAG	Lys	GAG	Glu
N33	AAT	Asn	AAT	Asn	GAT	Asp
P34	CCT	Pro	ACT	Thr	GCT	Ala
H35	CAC	His	AAC	Asn	GAC	Asp
T37	ACC	Thr	ACC	Thr	GCC	Ala
P59	CCA	Pro	ACA	Thr	GCA	Ala
K60	AAA	Lys	AAA	Lys	GAA	Glu
F61	TTC	Phe	ATC	Ile	GTC	Val
A62	GCA	Ala	ACA	Thr	GCA	Ala
V63	GTC	Val	ATC	Ile	GTC	Val
D83	GAT	Asp	AAT	Asn	GAT	Asp
V84	GTG	Val	ATG	Met	GTG	Val
S85	TCT	Ser	ACT	Thr	GCT	Ala
A86	GCG	Ala	ACG	Thr	GCG	Ala
Y89	TAT	Tyr	AAT	Asn	GAT	Asp
H90	CAT	His	AAT	Asn	GAT	Asp
I/L91	ATT	Ile	ATT	Ile	GTT	Val
P177	CCG	Pro	ACG	Thr	GCG	Ala
F178	TTT	Phe	ATT	Ile	GTT	Val
L179	CTC	Leu	ATC	Ile	GTC	Val
L180	CTG	Leu	ATG	Met	GTG	Val
A181	TTG	Leu	ATG	Met	GTG	Val
Q183	CAG	Gln	AAG	Lys	GAG	Glu
F183	TTT	Phe	ATT	Ile	GTT	Val
T184	ACT	Thr	ACT	Thr	GCT	Ala
Y203	TAT	Tyr	AAT	Asn	GAT	Asp
M204	ATG	Met	ATG	Met	GTG	Val
L235	TTG	Leu	ATG	Met	GTG	Val
N236	AAC	Asn	AAC	Asn	GAC	Asp
T237	ACT	Thr	ACT	Thr	GCT	Ala
P237	CCT	Pro	ACT	Thr	GCT	Ala
N238	AAT	Asn	AAT	Asn	GAT	Asp
H238	CAC	His	AAC	Asn	GAC	Asp
A238	GCT	Ala	ACT	Thr	GCT	Ala
S239	TCT	Ser	ACT	Thr	GCT	Ala
Q238	CAG	Gln	AAG	Lys	GAG	Glu
K239	AAA	Lys	AAA	Lys	GAA	Glu
L247	CTT	Leu	ATT	Ile	GTT	Val
N248	AAC	Asn	AAC	Asn	GAC	Asp
H248	CAT	His	AAT	Asn	GAT	Asp
F249	TTC	Phe	ATC	Ile	GTC	Val
M250	ATG	Met	ATG	Met	GTG	Val
G251	GGT	Gly	AGT	Ser	GGT	Gly
V251	GTC	Val	ATC	Ile	GTC	Val

TABLE 13 continued (II)

Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
CAG	Gln	TAG	Stop	AAG	Lys
CAT	His	TAT	Tyr	AAT	Asn
CCT	Pro	TCT	Ser	CAT	His
CAC	His	TAC	Tyr	CAC	His
CCC	Pro	TCC	Ser	AAC	Asn
CCA	Pro	TCA	Ser	CAA	Gln
CAA	Gln	TAA	Stop	AAA	Lys
CTC	Leu	TTC	Phe	TAC	Tyr
CCA	Pro	TCA	Ser	GAA	Glu
CTC	Leu	TTC	Phe	GAC	Asp
CAT	His	TAT	Tyr	GAT	Asp
CTG	Leu	TTG	Leu	GAG	Glu
CCT	Pro	TCT	Ser	TAT	Tyr
CCG	Pro	TCG	Ser	GAG	Glu
CAT	His	TAT	Tyr	TAT	Tyr
CAT	His	TAT	Tyr	CAT	His
CTT	Leu	TTT	Phe	AAT	Asn
CCG	Pro	TCG	Ser	CAG	Gln
CTT	Leu	TTT	Phe	TAT	Tyr
CTC	Leu	TTC	Phe	CAC	His
CTG	Leu	TTG	Leu	CAG	Gln
CTG	Leu	TTG	Leu	TAG	Stop
CAG	Gln	TAG	Stop	CAG	Gln
CTT	Leu	TTT	Phe	TAT	Tyr
CCT	Pro	TCT	Ser	AAT	Asn
CAT	His	TAT	Tyr	TAT	Tyr
CTG	Leu	TTG	Leu	AAG	Lys
CTG	Leu	TTG	Leu	TAG	Stop
CAC	His	TAC	Tyr	AAC	Asn
CCT	Pro	TCT	Ser	AAT	Asn
CCT	Pro	TCT	Ser	CAT	His
CAT	His	TAT	Tyr	AAT	Asn
CAC	His	TAC	Tyr	CAC	His
CCT	Pro	TCT	Ser	GAT	Asp
CCT	Pro	TCT	Ser	TAT	Tyr
CAG	Gln	TAG	Stop	CAG	Gln
CAA	Gln	TAA	Stop	AAA	Lys
CTT	Leu	TTT	Phe	CAT	His
CAC	His	TAC	Tyr	AAC	Asn
CAT	His	TAT	Tyr	CAT	His
CTC	Leu	TTC	Phe	TAC	Tyr
CTG	Leu	TTG	Leu	AAG	Lys
CGT	Arg	TGT	Cys	GAT	Asp
CTC	Leu	TTC	Phe	GAC	Asp

TABLE 13 continued (III)

Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
AGG	Arg	ACG	Thr	ATG	Met
AGT	Ser	ACT	Thr	ATT	Ile
CGT	Arg	CCT	Pro	CTT	Leu
CGC	Arg	CCC	Pro	CTC	Leu
AGC	Ser	ACC	Thr	ATC	Ile
CGA	Arg	CCA	Pro	CTA	Leu
AGA	Arg	ACA	Thr	ATA	Ile
TGC	Cys	TCC	Ser	TTC	Phe
GGA	Gly	GCA	Ala	GTA	Val
GGC	Gly	GCC	Ala	GTC	Val
GGT	Gly	GCT	Ala	GTT	Val
GGG	Gly	GCG	Ala	GTG	Val
TGT	Cys	TCT	Ser	TTT	Phe
GGG	Gly	GCG	Ala	GTG	Val
TGT	Cys	TCT	Ser	TTT	Phe
CGT	Arg	CCT	Pro	CTT	Leu
AGT	Ser	ACT	Thr	ATT	Ile
CGG	Arg	CCG	Pro	CTG	Leu
TGT	Cys	TCT	Ser	TTT	Phe
CGC	Arg	CCC	Pro	CTC	Leu
CGG	Arg	CCG	Pro	CTG	Leu
TGG	Trp	TCG	Ser	TTG	Leu
CGG	Arg	CCG	Pro	CTG	Leu
TGT	Cys	TCT	Ser	TTT	Phe
AGT	Ser	ACT	Thr	ATT	Ile
TGT	Cys	TCT	Ser	TTT	Phe
AGG	Arg	ACG	Thr	ATG	Met
TGG	Trp	TCG	Ser	TTG	Leu
AGC	Ser	ACC	Thr	ATC	Ile
AGT	Ser	ACT	Thr	ATT	Ile
CGT	Arg	CCT	Pro	CTT	Leu
AGT	Ser	ACT	Thr	ATT	Ile
CGC	Arg	CCC	Pro	CTC	Leu
GGT	Gly	GCT	Ala	GTT	Val
TGT	Cys	TCT	Ser	TTT	Phe
CGG	Arg	CCG	Pro	CTG	Leu
AGA	Arg	ACA	Thr	ATA	Ile
CGT	Arg	CCT	Pro	CTT	Leu
AGC	Ser	ACC	Thr	ATC	Ile
CGT	Arg	CCT	Pro	CTT	Leu
TGC	Cys	TCC	Ser	TTC	Phe
AGG	Arg	ACG	Thr	ATG	Met
GGT	Gly	GCT	Ala	GTT	Val
GGC	Gly	GCC	Ala	GTC	Val

TABLE 13 continued (IV)

Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
CCA	Pro	CCG	Pro	CCC	Pro	CCT	Pro
CAA	Gln	CAG	Gln	CAC	His	CAT	His
ACA	Thr	ACG	Thr	ACC	Thr	ACT	Thr
CCA	Pro	CCG	Pro	CCC	Pro	CCT	Pro
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
TTA	Leu	TTG	Leu	TTC	Phe	TTT	Phe
GCA	Ala	GCG	Ala	GCC	Ala	GCT	Ala
GTA	Val	GTG	Val	GTC	Val	GTT	Val
GAA	Glu	GAG	Glu	GAC	Asp	GAT	Asp
GTA	Val	GTG	Val	GTC	Val	GTT	Val
TCA	Ser	TCG	Ser	TCC	Ser	TCT	Ser
GCA	Ala	GCG	Ala	GCC	Ala	GCT	Ala
TAA	Stop	TAG	Stop	TAC	Tyr	TAT	Tyr
CAA	Gln	CAG	Gln	CAC	His	CAT	His
ATA	Ile	ATG	Met	ATC	Ile	ATT	Ile
CCA	Pro	CCG	Pro	CCC	Pro	CCT	Pro
TTA	Leu	TTG	Leu	TTC	Phe	TTT	Phe
CTA	Leu	CTG	Leu	CTC	Leu	CTT	Leu
CTA	Leu	CTG	Leu	CTC	Leu	CTT	Leu
TTA	Leu	TTG	Leu	TTC	Phe	TTT	Phe
CAA	Gln	CAG	Gln	CAC	His	CAT	His
TTA	Leu	TTG	Leu	TTC	Phe	TTT	Phe
ACA	Thr	ACG	Thr	ACC	Thr	ACT	Thr
TAA	Stop	TAG	Stop	TAC	Tyr	TAT	Tyr
ATA	Ile	ATG	Met	ATC	Ile	ATT	Ile
TTA	Leu	TTG	Leu	TTC	Phe	TTT	Phe
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
ACA	Thr	ACG	Thr	ACC	Thr	ACT	Thr
CCA	Pro	CCG	Pro	CCC	Pro	CCT	Pro
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
CAA	Gln	CAG	Gln	CAC	His	CAT	His
GCA	Ala	GCG	Ala	GCC	Ala	GCT	Ala
TCA	Ser	TCG	Ser	TCC	Ser	TCT	Ser
CAA	Gln	CAG	Gln	CAC	His	CAT	His
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
CTA	Leu	CTG	Leu	CTC	Leu	CTT	Leu
AAA	Lys	AAG	Lys	AAC	Asn	AAT	Asn
CAA	Gln	CAG	Gln	CAC	His	CAT	His
TTA	Leu	TTG	Leu	TTC	Phe	TTT	Phe
ATA	Ile	ATG	Met	ATC	Ile	ATT	Ile
GGA	Gly	GGG	Gly	GGC	Gly	GGT	Gly
GTA	Val	GTG	Val	GTC	Val	GTT	Val

TABLE 14 Amino acid mutations at target sites in rt

Target	Mutation
K32	M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L
N33	D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R
P34	S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F
H35	I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G
T37	W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S
P59	S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F
K60	M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L
F61	P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M
A62	R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V
V63	A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y
D83	C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N
V84	A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y
S85	T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P
A86	R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V
Y89	V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W
H90	I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G
I/L91	K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H
P177	S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F
F178	P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M
L179	K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I
L180	K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I
A181	R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V
Q183	E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C
F183	P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M
T184	W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S
Y203	V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W
M204	F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K
L235	K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I
N236	D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R
T237	W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S
P237	S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F
N238	D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R
H238	I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G
A238	R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V
S239	T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P
Q238	E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C
K239	M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L
L247	K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I
N248	D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R
H248	I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G

Target	Mutation
F249	P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M
M250	F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K
G251	H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E
V251	A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y

TABLE 15 Summary of HBV mutations in Patient F treated with ADV

Sample Name	Genotype	Polymerase*	Surface
CAP 01564808	A	**rtL157L/M** **rtA181V** **rtV207I** **rtN236T**	**sF83S** **sL173F** **sW199L**

* Nomenclature according to Stuyver *et al.*, 2001, *supra.*

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 16 Summary of HBV mutations in Patient G treated with ADV

Sample Name	Genotype	Polymerase*	Surface
KAN 02510355	C	**rtL80V** rtP109S rtI163V **rtM204I** rtL229M **rtN/H/A/S/Q238K**	sI126T sK160R sS174N sA184V **sW196L** sS210N **sF/C220L** sY221C

* Nomenclature according to Stuyver *et al.*, 2001, *supra.*

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 17 Summary of HBV mutations in Patient H treated with ADV

Sample Name	Genotype	Polymerase*	Surface
LAV0303	D	rtS78S/T **rtN118N/S** **rtN139N/K** **rtV142E** **rtA181A/T** rtI204M rtQ/P/S/Stop215Q **rtE218K/E** **rtN238N/H**	**sC69Stop/C** **sC76Y** **sI110V/I** **sY134N** **sW172Stop/W** **sW196Stop** **sS207R**

* Nomenclature according to Stuyver *et al.*, 2001, *supra.*

** Mutations in bold have not been detected in reference HBV genotypes, mutations not in bold are changes from the previous sample that are present in reference genotypes.

TABLE 18 *In vitro* drug susceptibility of the HBV reference laboratory strain and patient-derived HBV isolate

	***In vitro* Susceptibility, IC_{50} (fold change from wild-type)**		
	Real-time PCR	**Southern Blot**	
Mutation	**Adefovir**	**Adefovir**	**Lamivudine**
Wild-type (pPC)	1	1	1
rt N236T/N238D	23	NA[1]	NA[1]
rt A181T/N236T/N238D	5.1	7.3	>100
rt L180M/M204V[2]	NT[5]	0.9	>2500

[1] NA, not analyzed.

[2] Data from Delaney *et al.*, 2001, *supra*

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features

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CLAIMS

1. An isolated HBV variant wherein said variant comprises a nucleoside mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to said DNA polymerase and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof

2. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV.

3. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to both LMV.

4. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to TFV.

5. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to FTC.

6. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and LMV.

7. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and TFV.

8. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to LMV and TFV.

9. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and FTC.

10. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to LMV and FTC.

11. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to TFV and FTC.

12. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and LMV and TFV.

13. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and LMV and FTC.

14. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to FTC and LMV and TFV.

15. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and FTC and TFV.

16. The isolated HBV variant of Claim 1 wherein said variant exhibits decreased sensitivity to ADV and LMV and TFV and FTC.

17. The isolated HBV variant of any one of Claims 1 to 16 wherein said variant exhibits reduced interactivity to an immunological reagent specific to HBsAg.

18. The isolated HBV variant of Claim 1 wherein said variant comprises a mutation in domain F of the HBV DNA polymerase thereby conferring an altered amino acid sequence to the sequence set forth in Formula I [SEQ ID NO:1]:

FORMULA I

L, X_1, X_2, D, W, G, P, C, X_3, X_4, H, G, X_5, H, X_6, I, R, X_7, P, R, T, P, X_8, R, V, X_9, G, G, V, F, L, V, D, K, N, P, H, N, T, X_{10}, E, S, X_{11}, L, X_{12}, V, D, F, S, Q, F, S, R, G, X_{13}, X_{14}, X_{15}, V, S, W, P, K, F, A, V, P, N, L, X_{16}, S, L, T, N, L, L, S*

wherein:

X_1 is L or R or I;
X_2 is E or D;
X_3 is T or D or A or N or Y;
X_4 is E or D;
X_5 is E or K or Q;
X_6 is H or R or N;
X_7 is I or T;
X_8 is A or S;
X_9 is T or R;
X_{10} is A or T or S;
X_{11} is R or T;
X_{12} is V or G;
X_{13} is S or I or T or N or V;
X_{14} is T or S or H or Y;
X_{15} is R or H or K or Q;
X_{16} is Q or P;

and wherein S* is designated as amino acid 74.

19. The isolated HBV variant of Claim 1 wherein said variant comprises a mutation in any one of domains A through E thereby conferring an altered amino acid sequence to the sequence set forth in Formula II [SEQ ID NO:2]:

FORMULA II

S X_1 L S W L S L D V S A A F Y H X_2 P L H P A A M P H L L X_3 G S S G L X_4 R Y V A R L S S X_5 S X_6 X_7 X N X X_8 Q X X_9 X_{10} X X X X_{11} L H X_{12} X_{13} C S R X_{14} L Y V S L X_{15} L L Y X_{16} T X_{17} G X_{18} K L H L X_{19} X_{20} H P I X_{21} L G F R K X_{22} P M G X_{23} G L S P F L L A Q F T S A I X_{24} X_{25} X_{26} X_{27} X_{28} R A F X_{29} H C X_{30} X_{31} F X_{32} Y M* D D X_{33} V L G A X_{34} X_{35} X_{36} X_{37} H X_{38} E X_{39} L X_{40} X_{41} X_{42} X_{43} X_{44} X_{45} X_{46} L L X_{47} X_{48} G I H L N P X_{49} K T K R W G Y S L N F M G Y X_{50} I G

wherein:

X is any amino acid

X_1 is N or D;

X_2 is I or P;

X_3 is I or V;

X_4 is S or D;

X_5 is T or N;

X_6 is R or N;

X_7 is N or I;

X_8 is N or Y or H;

X_9 is H or Y;

X_{10} is G or R;

X_{11} is D or N;

X_{12} is D or N;

X_{13} is S or Y;

X_{14} is N or Q;

X_{15} is L or M;

X_{16} is K or Q;

X_{17} is Y or F;

X_{18} is R or W;

X_{19} is Y or L;

X_{20} is S or A;

X_{21} is I or V;

X_{22} is I or L;

X_{23} is V or G;

X_{24} is C or L;

X_{25} is A or S;

X_{26} is V or M;

X_{27} is V or T;

X_{28} is R or C;

X_{29} is F or P;

X_{30} is L or V;

X_{31} is A or V;

X_{32} is S or A;

X_{33} is V or L or M;

X_{34} is K or R;

X_{35} is S or T;

X_{36} is V or G;

X_{37} is Q or E;

X_{38} is L or S or R;

X_{39} is S or F;

X_{40} is F or Y;

X_{41} is T or A;

X_{42} is A or S;

X_{43} is V or I;

X_{44} is T or C;

X_{45} is N or S;

X_{46} is F or V;

X_{47} is S or D;

X_{48} is L or V;

X_{49} is N or Q;

X_{50} is V or I; and

M^* is amino acid 204;

and wherein the first S is designated as amino acid 75.

20. The isolated HBV variant of Claim 18 or 19 wherein said variant further comprises an altered HBsAg.

21. An isolated HBV variant comprising a mutation in the nucleotide sequence encoding HBsAg resulting in an amino acid addition, substitution and/or deletion in said HBsAg in a region corresponding to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof.

22. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV.

23. The isolated HBV variant of Claim 21 wherein said variants exhibits decreased sensitivity to LMV.

24. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to TFV.

25. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to FTC.

26. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased

sensitivity to ADV and LMV.

27. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to LMV and TFV.

28. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV and FTC.

29. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to LMV and FTC.

30. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to TFV and FTC.

31. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV and TFV.

32. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV and LMV and TFV.

33. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV and LMV and FTC.

34. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to FTC and LMV and TFV.

35. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV and FTC and TFV.

36. The isolated HBV variant of Claim 21 wherein said variant exhibits decreased sensitivity to ADV and LMV and TFV and FTC.

37. The isolated HBV variant of Claim 21 wherein an antibody specific for a wild-type HBsAg exhibits a reduced capacity to neutralize said HBV variant and wherein said HBV variant is selected by exposure of a subject to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, in single or combinations or sequential therapy.

38. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtS21A, rtL122F, rtN124H, rtT28N, rtP130Q, rtD131N and rtY135C or a combination thereof or an equivalent mutation.

39. The isolated HBV variant of Claim 1 or 37 comprising a muation in the HBV DNA polymerase selected from rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M or a combination thereof or an equivalent mutation.

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40. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D or a combination thereof or an equivalent mutation.

41. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtH90D and rtL/F108L or a combination thereof or an equivalent mutation.

42. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtL157L/M, rtA181V and rtV207I or a combination thereof or an equivalent mutation.

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43. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV

DNA polymerase selected from rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K or a combination thereof or an equivalent mutation.

44. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the surface antigen selected from sP120T, sM125F and sT127A or a combination thereof or an equivalent mutation.

45. The isolated HBV variant of Claim1 or 37 comprising a mutation in the surface anitgen selected from sT118R, sM133T, sF134V, sI195M, sS207R and sY225Y/C or a combination thereof or an equivalent mutation.

46. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the surface antigen selected from sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop or a combination thereof or an equivalent mutation.

47. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV surface antigen selected from s181M and sP214Q or a combination thereof or an equivalent mutation.

48. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV surface antigen selected from sF83S, sL173F and sW199L or a combination thereof or an equivalent mutation.

49. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV surface antigen selected from sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C or a combination thereof or an equivalent mutation.

50. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV surface antigen selected from sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R or a combination thereof or an equivalent mutation.

51. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtK32, rtN33, rtP34, rtH35 and rtT37 or a combination thereof or an equivalent mutation.

52. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtP59, rtK60, rtF61, rtA62 and rtV63 or a combination thereof or an equivalent mutation.

53. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91 or a combination thereof or an equivalent mutation.

54. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184 or a combination thereof or an equivalent mutation.

55. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtM204 and rtY203 or a combination thereof or an equivalent mutation.

56. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rt235, rt236, rt237, rt238 and rt239 or a combination thereof or an equivalent mutation.

57. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rt247, rt248, rt249, rt250 and rt251 or a combination thereof or an equivalent mutation.

58. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion; N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;

P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;

A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion.

59. A method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV or optionally other nucleoside analogs or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and A through E or a region proximal thereto of said DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV.

60. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to

ADV.

61. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to LMV.

62. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to TFV.

63. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to FTC.

64. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV and LMV.

65. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV and TFV.

66. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to LMV and TFV.

67. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV and FTC.

68. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to TFV and FTC.

69. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to LMV and FTC.

70. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV and LMV and TFV.

71. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV

and LMV and FTC.

72. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to LMV and TFV and FTC.

73. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV and FTC and TFV.

74. The method of Claim 59 wherein the HBV exhibits reduced sensitivity to ADV and LMV and TFV and FTC.

75. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtS21A, rtL122F, rtN124H, rtT28N, rtP130Q, rtD131N and rtY135C or a combination thereof or an equivalent mutation.

76. The isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a muation in the HBV DNA polymerase selected from rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M or a combination thereof or an equivalent mutation.

77. The isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D or a combination thereof or an equivalent mutation.

78. The isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A or a combination thereof or an equivalent mutation.

79. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtH90D and rtL/F108L or a combination thereof or an equivalent mutation.

80. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected rtL157L/M, rtA181V and rtV207I or a combination thereof or an equivalent mutation.

81. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K or a combination thereof or an equivalent mutation.

82. The isolated HBV variant of Claim 1 or 37 comprising a mutation in the DNA polymerase selected from rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H or a combination thereof or an equivalent mutation.

83. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the surface antigen gene selected from sP120T, sM125F and sT127A or a combination thereof or an equivalent mutation.

84. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV Surface antigen gene selected from sT118R, sM133T, sF134V, sI195M, sS207R and sY225Y/C or a combination thereof or an equivalent mutation.

85. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the surface antigen selected from sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop or a combination thereof or an equivalent mutation.

86. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA surface antigen selected from sN40S, sC69Stop, sM75I,

sL88P, sT118A, sW182Stop, sW196L, sY206H an sY225F or a combination thereof or an equivalent mutation.

87. An isolated DNA molecule from an HBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA surface antigen selected from s181M and sP214Q or a combination thereof or an equivalent mutation.

88. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA surface antigen selected from sF83S, sL173F and sW199L or a combination thereof or an equivalent mutation.

89. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA surface antigen selected from sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C or a combination thereof or an equivalent mutation.

90. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA surface antigen selected from sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R or a combination thereof or an equivalent mutation.

91. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtK32, rtN33, rtP34, rtH35 and rtT37 or a combination thereof or an equivalent mutation.

92. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtP59, rtK60, rtF61, rtA62 and rtV63 or a combination thereof or an equivalent mutation.

93. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtD83, rtV84, rtS85, rtA86, rtY89,

rtH90 and rtI/L91 or a combination thereof or an equivalent mutation.

94. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184 or a combination thereof or an equivalent mutation.

95. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rtM204 and rtY203 or a combination thereof or an equivalent mutation.

96. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rt235, rt236, rt237, rt238 and rt239 or a combinations thereof or an equivalent mutation.

97. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymerase selected from rt247, rt248, rt249, rt250 and rt251 or a combinations thereof or an equivalent mutation.

98. An isolated DNA molecule from anHBV variant of Claim 1 or 37 comprising a mutation in the HBV DNA polymeraes selected from
K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;

V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;

G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion.

99. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtS21A, rtL122F, rtN124H, rtT28N, rtP130Q, rtD131N and rtY135C or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

100. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

101. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and

LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

102. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and optionally other nucleoside analogs.

103. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtH90D and rtL/F108L or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

104. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtL157L/M, rtA181V and rtV207I or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and

optionally other nucleoside analogs.

105. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

106. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

107. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sP120T, sM125F and sT127A or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally

other nucleoside analogs.

108. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sT118R, sM133T, sF134V, sI195M, sS207R and sY225Y/C or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

109. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

110. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182Stop, sW196L, sY206H an sY225F or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

111. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of s181M and sP214Q or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

112. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sF83S, sL173F and sW199L or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

113. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

114. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

115. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtK32, rtN33, rtP34, rtH35 and rtT37 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

116. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtP59, rtK60, rtF61, rtA62 and rtV63 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

117. A method for determining whether an HBV strain exhibits reduced sensitivity

to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

118. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

119. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rtM204 and rtY203 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV,and optionally other nucleoside analogs.

120. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA

from said HBV and screening for a mutation wherein the presence of rt235, rt236, rt237, rt238 and rt239 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

121. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of rt247, rt248, rt249, rt250 and rt251 or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside analogs.

122. A method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation wherein the presence of
K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;

D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/YV/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;

M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and optionally other nucleoside analogs.

123. A method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and optionally other nucleoside or nucleotide analogs or other anti-HBV agents, said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in a plasmid vector and then transfecting said cells with said construct;

contacting said cells, before, during and/or after transfection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

124. A method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV, and optionally other nucleoside or nucleotide analogs or other anti-HBV agents, said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct;

contacting said cells, before, during and/or after infection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

125. The method of Claim 123 or 124 wherein the HBV genome is stably integrated into the cells' genome.

126. An agent identified by the method of any one of Claims 123 to 124.

127. Use of an HBV variant according to any one of Claims 1 to 59 or a component thereof in the rational design of an anti-HBV agent.

128. Use according to Claim 126 wherein the rational design comprises microarray analysis.

129. Use according to Claim 126 wherein the rational design is based on the crystal structure or NMR structure of a viral component.

130. A vaccine comprising an antigenic component of the HBV variant of any one of Claims 1 to 59 or an antigenic component thereof or an antibody thereto.

131. The vaccine of Claim 130 wherein the antigenic component is an HBsAg or PreS1 or PreS2.

132. The vaccine of Claim 130 wherein the antigenic component is a defective HBV variant.

133. The vaccine of Claim 130 comprising an antibody to HBsAg or PreS1 or PreS2

134. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtS21A, rtL122F, rtN124H, rtT28N, rtP130Q, rtD131N and rtY135C.

135. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rt/N/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V and rtI235I/M.

136. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F.

137. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D.

138. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A.

139. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtH90D and rtL/F108L.

140. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtL157L/M, rtA181V and rtV207I.

141. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtL80V, rtP109S, rtI163V, rtL229M and rtN/H/A/S/Q238K.

142. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E and rtN238N/H.

143. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sP120T, sM125F and sT127A.

144. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sT118R, sM133T, sF134V, sI195M, sS207R and sY225Y/C.

145. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sS126T, sM133L/M, sS143S/T, sD144A, sG145A and sW172Stop.

146. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sN40S, sC69Stop, sM75I, sL88P, sT118A, sW182Stop, sW196L, sY206H an sY225F.

147. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from s181M and sP214Q.

148. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sF83S, sL173F and sW199L.

149. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C.

150. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from sC69Stop/C, sC76Y, sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R.

151. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtK32, rtN33, rtP34, rtH35 and rtT37.

152. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtP59, rtK60, rtF61, rtA62 and rtV63.

153. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 and rtI/L91.

154. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184.

155. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rtM204 and rtY203.

156. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rt235, rt236, rt237, rt238 and rt239.

157. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from rt247, rt248, rt249, rt250 and rt251.

158. The vaccine of Claim 130 wherein the HBV or its antigenic compound is from an HBV variant having a mutation selected from,
K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;

H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion.

159. A computer product for assessing the likely usefulness of a viral variant or biological sample comprising same for determining an appropriate therapeutic protocol in a subject, said product comprising:

(1) code that receives as input index values (I_{VS}) for at least two features associated with said viral agents or biological sample comprising same, wherein said features are selected from:

(a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
(b) an altered DNA polymerase from wild-type HBV;
(c) an altered surface antigen from wild-type HBV;
(d) morbidity or recovery potential of a patient; or
(e) altered replication capacity (increased or decreased);

(2) code that adds said I_{VS} to provide a sum corresponding to a potency value (P_V) for said viral variants or biological samples; and

(3) a computer readable medium that stores the codes.

160. A computer for assessing the likely usefulness of a viral variant or biological sample comprising same in a subject, wherein said computer comprises:

(1) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise I_{VS} for at least two features associated with said viral variant or biological sample; wherein said features are selected from:-

(a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;

(b) an altered DNA polymerase from wild-type HBV;

(c) an altered surface antigen from wild-type HBV;

(d) morbidity or recovery potential of a patient; or

(e) altered replication capacity (increased or decreased);

(2) a working memory for storing instructions for processing said machine-readable data;

(3) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine readable data to provide a sum of said I_{VS} corresponding to a P_V for said compound(s); and

(4) an output hardware coupled to said central processing unit, for receiving said P_V.

161. A composition comprising an agent capable of directly or indirectly inhibiting an HBV variant as defined in any one of Claims 1 to 59, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

162. The composition of Claim 161 wherein the agent is a recombinant protein from said HBV variant.

163. The composition of Claim 161 wherein the recombinant protein is HBsAg or PreS1 or PreS2.

164. The composition of Claim 161 wherein the agent is capable of inhibiting an HBV variant polymerase.

165. The composition of Claim 161 wherein the agent is identified by natural product screening or rational drug design.

166. The composition of Claim 161 wherein the agent is a defective HBV variant.

167. The composition of Claim 161 wherein the agent is an antibody directed to an HBV compound.

168. The composition of Claim 161 wherein the agent is a ribozyme, antisense molecule or sense molecule relative to an HBV gene.

169. A method according to Claims 37 to 57 wherein a virus related to HBV from the family of hepdanviruses such as WHV or DHBV exhibits reduced sensitivity to a nucleoside analog, said method comprising isolating DNA or corresponding mRNA from said HBV, or DHBV or WHV and screening for a mutation or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV.

170. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

171. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA

or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sP120T, sM125T and sT127A selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

172. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sT118R, sM133T, sF134V sI195M, sS207R and sY225Y/C selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

173. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sS126T, sM133L/M, sS143S/T, sD144A sG145A and sW172Stop selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

174. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sN40S, and sC69 Stop, sM75I, sL88P, sT118A, sW182stop, sW196L, sY206H and sY225F selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

175. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sF83S, sL173F and sW199L selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

176. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and

LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

177. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

178. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

179. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtS21A, rtL122F, rtN124H, rtH126R,

rtT128N, rtP130Q, rtD131N, rtY135C selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

180. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtN/S/T/I/V53D, rtY126Q, rtL180M, rtS202G, rtI204V, rtI235I/M selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

181. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtN53D, rtY54H, rtS57P, rtL91I, rtS116P, rtF122L, rtY124H, rtV134D, rtY141Y/F, rtL145M, rtF151F/Y, rtA181T, rtK212R, rtL217R, rtS219A, rtN236T and rtN238D selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

182. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtS78T, rtV84M, rtY126C, rtV191I, rtM204I and rtV214A selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

183. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtH90D and rtL/F108L selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

184. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtL157L/M, rtA181V, rtV207I; and rtN236T selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing

interactivity of said antibodies to said mutated surface antigen.

185. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtL80V, rtP109S, rtI163V, rtM204I, rtL229M and rtN/H/A/S/Q238K selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

186. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtS78S/T, rtN118N/S, rtN139N/K, rtV142E, rtA181A/T, rtI204M, rtQ/P/S/Stop215Q, rtE218K/E, and rtN238N/H selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

187. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtK32, rtN33, rtP34, rtH35 and rtT37 selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV,

LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

188. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtP59, rtK60, rtF61, rtA62 and rtV63 elected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

189. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtD83, rtV84, rtS85, rtA86, rtY89, rtH90 selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

190. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the

presence of such a mutation is selected from rtP177, rtF178, rtL179, rtL180, rtA181, rtQ182, rtF183 and rtT184 selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

191. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rtM204 and rtY203 selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

192. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rt235, rt236, rt237, rt238 and rt239 selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

193. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA

or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation is selected from rt247, rt248, rt249, rt250 and rt251 selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

194. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV surface antigen and/or HBV DNA polymerase genes wherein the presence of such a mutation, K32M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
N33D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
P34S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
H35I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
T37W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P59S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
K60M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
F61P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
A62R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
V63A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
D83C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/deletion;
V84A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion;
S85T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
A86R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Y89V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
H90I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
I/L91K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/deletion;
P177S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;

F178P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
L179K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
L180K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
A181R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
Q183E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
F183P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
T184W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
Y203V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/deletion;
M204F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
L235K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N236D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
T237W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/deletion;
P237S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/deletion;
N238D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H238I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
A238R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/deletion;
S239T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/deletion;
Q238E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/deletion;
K239M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/deletion;
L247K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/deletion;
N248D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/V/A/R/deletion;
H248I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/deletion;
F249P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/M/deletion;
M250F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/G/H/I/L/K/deletion;
G251H/I/L/K/M/F/P/S/T/W/Y/V/A/R/N/D/C/Q/E/deletion; and
V251A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/S/T/W/Y/deletion, is selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

195. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV and is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

196. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation is selected from sP120T, sM125T and sT127A selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

197. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected from sT118R, sM133T, sF134V sI195M, sS207R and sY225Y/C selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

198. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected from sS126T, sM133L/M, sS143S/T, sD144A sG145A and sW172Stop selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

199. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected from sN40S, and sC69 Stop, sM75I, sL88P, sT118A, sW182stop, sW196L, sY206H and sY225F selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

200. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected from sF83S, sL173F and sW199L selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen

201. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected from sI126T, sK160R, sS174N, sA184V, sW196L, sS210N, sF/C220L and sY221C selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

202. A method for determining the potential for an HBV to exhibit reduced interactivity to antibody to HBV surface antigen, said method comprising isolated protein from said HBV encoding HBV surface antigen wherein the presence of such a mutation was selected sC69Stop/C, sC76Y sI110V/I, sY134N, sW172Stop/W, sW196Stop and sS207R selected during treatment with ADV, LMV, TFV, or FTC, or ADV and LMV, ADV and TFV, LMV and TFV, FTC and ADV, FTC and TFV, FTC and LMV, or ADV and LMV and TFV, or ADV and FTC and TFV, TFV and FTC and LMV, ADV and LMV and FTC, or ADV and FTC and LMV and TFV is an indication of the likelihood of reducing interactivity of said antibodies to said mutated surface antigen.

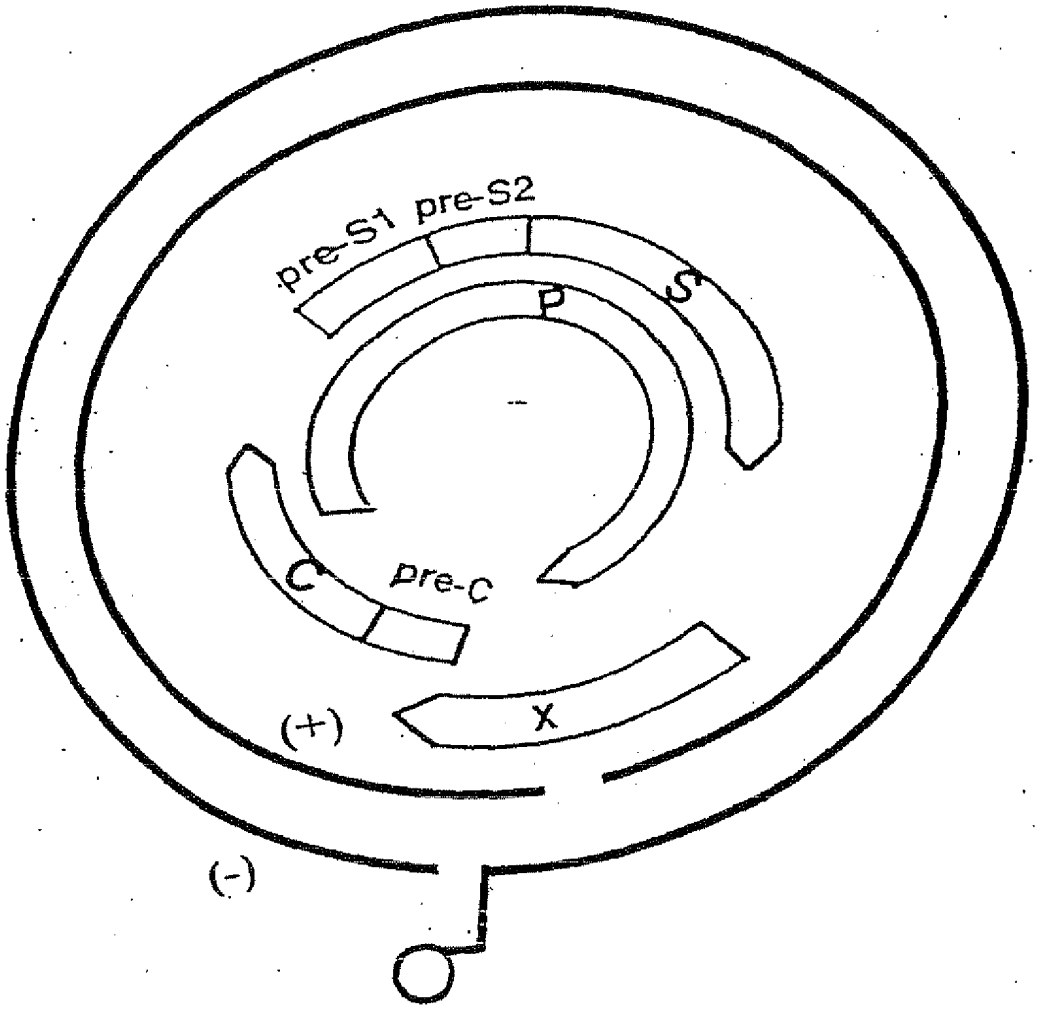

Figure 1

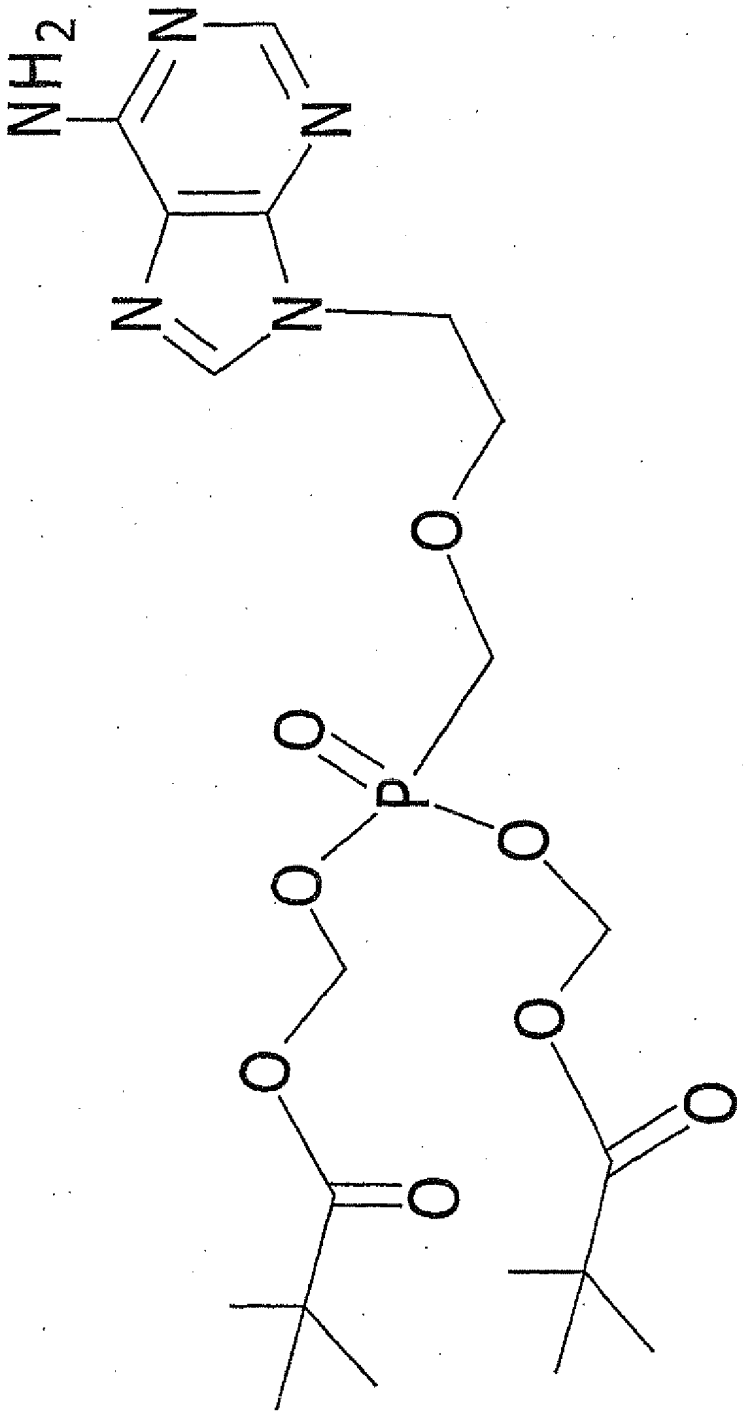

Figure 2

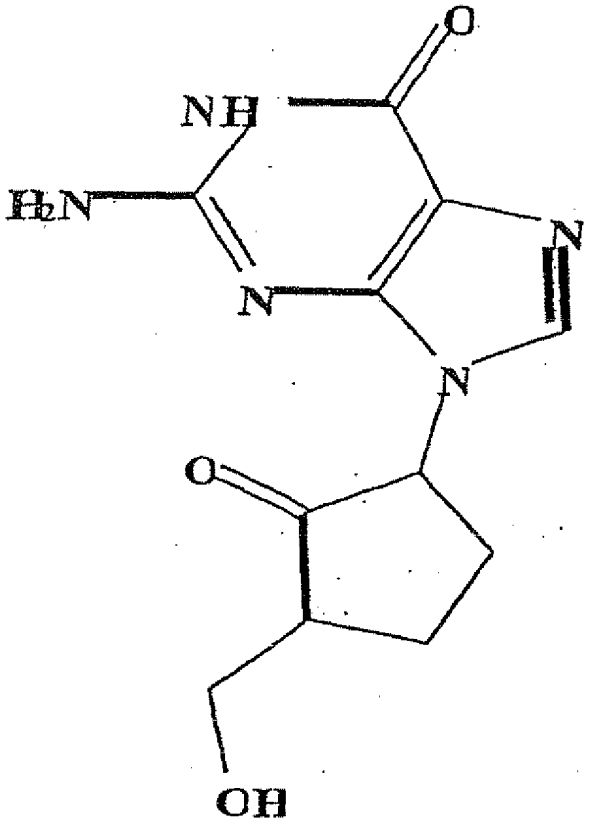

Figure 3

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ILA1 F, A-E      1                                  CAAAACTTGCCAGCAAATCCGCCTCCTCCATCYACCAATCGCCAGTCAGGAMGGCAGCCTACCCCGCTGTCTCCACCTTT   80   [SEQ ID NO:8]
ILA 2 F,A-E      1                            GCATACTACAAA-CTTTGCCAGCAAATCCGCCTCCTGCCTCCACCAATCGCCAGTCAGGAAGGCAGCCTACCCCGCTGTCTCCACCTTT   80   [SEQ ID NO:9]
ILA 3 F, A-E     1  ACTAGCTCAGGGCATACTACAAAACTTTGCCAGCAAATCCGCCTCCTGCCTCCACCAATCGCCAGTCAGGAAGGCAGCCTACCCCGCTGTCTCCACCTTT   88   [SEQ ID NO:9]
ILA 4 F,A-E      1                                  TGCCAGCAAATCCGCCTCCTCCATCTACCAATCGCCAGTCGGGACGGCAGCCTACCCCGCTGTCTCCACCTTT  100   [SEQ ID NO:10]
ILA1 F, A-E      1                                  CAAAACTTGCCAGCAAATCCGCCTCCTCCATCTACCAATCGCCAGTCGGGACGGCAGCCTACCCCGCTGTCTCCACCTTH   73   [SEQ ID NO:11]

ILA1 F, A-E     81  GAGAAACACTCATCCTCAGGCCATGCAGTGGAAYTCCACAACCTTCCACCAAACTCTGMAAGATCCCAGRGTGARAGGCCTGTATTTCCCTGCTGGTGGC  180
ILA 2 F,A-E     89  GAGAAACACTCATCCTCAGGCCATGCAGTGGAATTCCACAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTGTATTTCCCTGCTGGTGGC  188
ILA 3 F, A-E   101  GAGAAACACTCATCCTCAGGCCATGCAGTGGAATTCCACAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTGTATTTCCCTGCTGGTGGC  200
ILA 4 F,A-E     74  GAGAAACACTCATCCTCAGGCCATGCAGTGGAACTCCACAACCTTCCACCAAACTCTGAAAGATCCCAGGGTGAGMGGCCTGTATTTCCCTGCTGGTGGC  173

ILA1 F, A-E    181  TCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCA  280
ILA 2 F,A-E    189  TCCAGTTCAGGAACAGTAAACCCTGTTCTGACTACTGCCTCTCCCTTATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCA  288
ILA 3 F, A-E   201  TCCAGTTCAGGAACAGTAAACCCTGTTCTGACTACTGCCTCTCCCTTATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCA  300
ILA 4 F,A-E    174  TCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCA  273

ILA1 F, A-E    281  CATCAGGATTCCTMGGACCCCTKCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTC  380
ILA 2 F,A-E    289  CATCAGGATTCCTAGGACCCCTTCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTC  388
ILA 3 F, A-E   301  CATCAGGATTCCTAGGACCCCTTCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTC  400
ILA 4 F,A-E    274  CATCAGGATTCCTCGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTC  373

ILA1 F, A-E    381  TCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGT  480
ILA 2 F,A-E    389  TCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCCAACTTGTCCTGGT  488
ILA 3 F, A-E   401  TCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCCAACTTGTCCTGGT  500
ILA 4 F,A-E    374  TCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGT  473

ILA1 F, A-E    481  TATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGC  580
ILA 2 F,A-E    489  TATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGC  588
ILA 3 F, A-E   501  TATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGC  600
ILA 4 F,A-E    474  TATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGC  573

ILA1 F, A-E    581  CCGTTTGTCCTCTAATTCCAGGATCTTCAACCACCAGCGCGGGAMCATGCAGAACCTGCACGACTACTGCTCAAGGAACCTCTATGTATCCCTCCTGTTG  680
ILA 2 F,A-E    589  CCGTTTGTCCTCTAATTCCAGGATCCTCAACAACCAGCACGGGACCATGCCGGACCTGCATGACTACTGCTCAAGGAACCTCTATGTATCCCTCCTGTTG  688
ILA 3 F, A-E   601  CCGTTTGTCCTCTAATTCCAGGATCCTCAACAACCAGCACGGGACCATGCCGGACCTGCATGACTACTGCTCAAGGAACCTCTATGTATCCCTCCTGTTG  700
ILA 4 F,A-E    574  CCGTTTGTCCTCTAATTCCAGGATCTTCAACCACCAGCGCGGGAACATGCAGAACCTGCACGACTGCTGCTCAAGGAACCTCTATGTATCCCTCCTGTTG  673

ILA1 F, A-E    681  CTGTACCAAACCTTCGGACGGAAACTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCC  780
ILA 2 F,A-E    689  CTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCC  788
ILA 3 F, A-E   701  CTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCC  800
ILA 4 F,A-E    674  CTGTACCAAACCTTCGGACGGAAACTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCC  773
```

Figure 4

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ILA1 F, A-E     781 TGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTC  880   [SEQ ID NO:8]
ILA 2 F,A-E     789 TGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTC  888   [SEQ ID NO:9]
ILA 3 F, A-E    801 TGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTC  900   [SEQ ID NO:10]
ILA 4 F,A-E     774 TGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTC  873   [SEQ ID NO:11]

ILA1 F, A-E     881 TGTAYAGCAYCTTGAGTCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAACAAAACTAAAAGATGGGGTTACTCT  980
ILA 2 F,A-E     889 TGTACAGCATCTTGAGTCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAACAAAACAAAGAGATGGGGTTACTCT  988
ILA 3 F, A-E    901 TGTACAGCATCTTGAGTCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAACAAAACAAAGAGATGGGGTTACTCT 1000
ILA 4 F,A-E     874 TGTACAGCATCTTGAGTCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGCATACATTTAAACCCTAACAAAACTAAAAGATGGGGGTACTCT  973

ILA1 F, A-E     981 TTACATTTCATGGGNTATGTCATTGGATGTTATGGGTCATTGCCACAAGATCACATCATACAGAAAATCAAAGATGGTTT 1060
ILA 2 F,A-E     989 CTAAATTTTATGGGTTATGTCATTGGATGTTATGGGTCCTTG                                      1030
ILA 3 F, A-E   1001 CTAAATTTTATGGGTTATGTCATTGGATGTTATGGGTCCTTGCCACAAGAACACATCATACAAAAAATCAAAGAATG     1077
ILA 4 F,A-E     974 TTAAATTTCATGGGATATGTCATTGGATGGTATGGGG                                          1010
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Figure 4 (continued)

Patient A polymerase amino acid sequence alignment

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Pol Trans Pre  1          KLASKSASSIXQSPVRXAAYPAVSTFEKHSSSGHAVEXHNLPPNSXRSQXERPVFPCWWLQFRNSKPCSDYCLSHIVNLLEDWGPCAEHGEHH  93  [SEQ ID NO:12]
Pol Trans 2            HTTNFASKSASCLHQSPVRKAAYPAVSTFEKHSSSGHAVEFHNLPPNSARSQSERPVFPCWWLQFRNSKPCSDYCLSLIVNLLEDWGPCAEHGEHH  96  [SEQ ID NO:13]
Pol Trans 3    1   LAQGILQNFASKSASCLHQSPVRKAAYPAVSTFEKHSSSGHAVEFHNLPPNSARSQSERPVFPCWWLQFRNSKPCSDYCLSLIVNLLEDWGPCAEHGEHH 100  [SEQ ID NO:14]
Pol Trans 4    1          ASKSASSIYQSPVGTAAYPAVSTXEKHSSSGHAVELHNLPPNSERSQGERPVFPCWWLQFRNSKPCSDYCLSHIVNLLEDWGPCAEHGEHH  91  [SEQ ID NO:15]

Pol Trans Pre  94   IRIPRTPXRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVA 193
Pol Trans 2    97   IRIPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVA 196
Pol Trans 3    101  IRIPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVA 200
Pol Trans of 4 92   IRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVA 191

Pol Trans Pre  194  RLSSNSRIFNHQRGXMQNLHDYCSRNLYVSLLLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKS 293
Pol Trans 2    197  RLSSNSRILNNQHGTMPDLHDYCSRNLYVSLLLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKS 296
Pol Trans 3    201  RLSSNSRILNNQHGTMPDLHDYCSRNLYVSLLLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKS 300
Pol Trans 4    192  RLSSNSRIFNHQRGNMQNLHDCCSRNLYVSLLLLYQTFGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKS 291

Pol Trans Pre  294  VXHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIGC   336
Pol Trans 2    297  VQHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGCY  340
Pol Trans 3    301  VQHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGCY  344
Pol Trans 4    292  VQHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLNFMGYVIGWYG 336
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Figure 5

Patient A HBsAg Amino acid alignment

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HBsAg Trans of  Pre   1                                                                                    MENITSGFLGPLLVLQA  17  [SEQ ID NO:16]
HBsAg Trans of  2     1    PPPASTNRQSGRQPTPLSPPLRNTHPQAMQWNSTTFHQTLQDPRVRGLYFPAGGSSSGTVNPVLTTASPLSSIFSRIGDPALNMENITSGFLGPLLVLQA 100  [SEQ ID NO:17]
HBsAg Trans of  3     1    PPPASTNRQSGRQPTPLSPPLRNTHPQAMQWNSTTFHQTLQDPRVRGLYFPAGGSSSGTVNPVLTTASPLSSIFSRIGDPALNMENITSGFLGPLLVLQA 100  [SEQ ID NO:18]
HBsAg Trans of  4     1    PPPPSTNRQSGRQPTPLSPPXRNTHPQAMQWNSTTFHQTLKDPRVXGLYFPAGGSSSGTVNPVPTTASPISSIFSRIGDPALNMENITSGFLGPLLVLQA 100  [SEQ ID NO:19]

HBsAg Trans of  Pre   18   GFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTS 117
HBsAg Trans of  2     101  GFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTS 200
HBsAg Trans of  3     101  GFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTS 200
HBsAg Trans of  4     101  GFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTS 200

HBsAg Trans of  Pre   118  AGXCRTCTTTAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYSXLSPFLPLLP 217
HBsAg Trans of  2     201  TGPCRTCMTTAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYSILSPFLPLLP 300
HBsAg Trans of  3     201  TGPCRTCMTTAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYSILSPFLPLLP 300
HBsAg Trans of  4     201  AGTCRTCTTAAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYSILSPFLPLLP 300

HBsAg Trans of  Pre   218  IFFCLWVYI 226
HBsAg Trans of  2     301  IFFCLWVYI 309
HBsAg Trans of  3     301  IFFCLWVYI 309
HBsAg Trans of  4     301  IFFCLWAYI 309
```

Figure 6

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                10         20         30         40         50
S0                                                              [SEQ ID NO:20]
S6                                                              [SEQ ID NO:21]
S8                                                         T    [SEQ ID NO:22]
S12   TTTTGGGGAGCCCTCAGGCTCAGGGCATATTACAAACTCTGCCAGCAAAT      [SEQ ID NO:23]
S15                                TACAAACTTTGCCAGCAAAT      [SEQ ID NO:24]

                60         70         80         90         100
S0
S6
S8    GCCCCTTCTGCCTCCACCAATCGCCAGTCAGGAAGGCAGCCTACCCCGCT
S12   CCACCTCCTGCCTCCACCAATCGCCAGTCAGGAAGGCAGCCTACCCCGCT
S15   CCACCTCCTGCCTCCACCAATCGCCAGTCAGGAAGGCAGCCTACCCCGCT

                110        120        130        140        150
S0
S6
S8    GTCTCCACCTTTGAGAGACACTCATCCTCAGGCCATGCAGTGGAACTCAA
S12   GTCTCCACCTTTGAGAGACACTCATCCTCAGGCCATGCAGTGGAACTCAA
S15   GTCTCCACCTTTGAGAGACACTCATCCTCAGGCCATGCAGTGGAACTCAA

                160        170        180        190        200
S0
S6
S8    CAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAAAGGCCTGTATTTC
S12   CAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAAAGGCCTGTATTTC
S15   CAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAAAGGCCTGTATTTC

                210        220        230        240        250
S0
S6
S8    CCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGC
S12   CCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGC
S15   CCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGC

                260        270        280        290        300
S0
S6
S8    CTCTCACTCATCGTCAATCTTCTCGAGGATTGGGGTCCCTGCGCTGAACA
S12   CTCTCACTCATCGTCAATCTTCTCGAGGATTGGGGTCCCTGCGCTGAACA
S15   CTCTCACTCATCGTCAATCTTCTCGAGGATTGGGGTCCCTGCGCTGAACA
```

Figure 7

```
          310        320        330        340        350
S0
S6
S8   TGGAGAACATCACATCAGGACTCCTAGGACCCCTTCTCGTGTTACAGGCG
S12  TGGAGAACATCACATCAGGACTCCTAGGACCCCTTCTCGTGTTACAGGCG
S15  TGGAGAACATCACATCAGGACTCCTAGGACCCCTTCTCGTGTTACAGGCG

          360        370        380        390        400
S0                                        CGCAGAGTCTAGACTC
S6
S8   GGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTC
S12  GGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTC
S15  GGGTTTTTNTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTC

          410        420        430        440        450
S0   GTGGTGGACTTCTCTCAATTTTCGAGGGGGGACTACCGTGTGTCTTGGCC
S6
S8   GTGGTGGACTTCTCTCAATTTTCGAGGGGGGACTACCGTGTGTCTTGGCC
S12  GTGGTGGACTTCTCTCAATTTTCGAGGGGGGACTACCGTGTGTCTTGGCC
S15  GTGGTGGACTTCTCTCAATTTTCGAGGGGGGACTACCGTGTGTCTTGGCC

          460        470        480        490        500
S0   AAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCA
S6                                  TTACTCACCNACCTCCTGTCCTCCA
S8   AAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCA
S12  AAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCA
S15  AAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCA

          510        520        530        540        550
S0   ACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCT
S6   ACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCT
S8   ACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCT
S12  ACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCT
S15  ACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCT

          560        570        580        590        600
S0   CTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTGTC
S6   CTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTGTC
S8   CTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTGTC
S12  CTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTGTC
S15  CTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGCTCTACTGGACTGTC

          610        620        630        640        650
S0   AAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGC
S6   AAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGC
S8   AAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGC
S12  AAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGC
S15  AAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGC
```

Figure 7 (continued)

```
          660        670        680        690        700
S0    ACGGGACCATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTACGGT
S6    AGGGGACCATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTACGGT
S8    AGGGGACCATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTACGGT
S12   AGGGGACCATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTACGGT
S15   AGGGGACCATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTACGGT

          710        720        730        740        750
S0    TCCCTCATGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTC
S6    TCCCTCATGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTC
S8    TCCCTCATGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTC
S12   TCCCTCATGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTC
S15   TCCCTCATGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTC

          760        770        780        790        800
S0    CCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCA
S6    CCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCA
S8    CCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCA
S12   CCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCA
S15   CCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCA

          810        820        830        840        850
S0    GCCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGT
S6    GCCCGTTTCTCATGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGT
S8    GCCCGTTTCTCATGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGT
S12   GCCCGTTTCTCATGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGT
S15   GCCCGTTTCTCATGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGT

          860        870        880        890        900
S0    AGGGCTTTCCCCCACTGTCTGGCTTTTAGTTATATGGATGATGTGGTATT
S6    AGGGCTTTCCCCCACTGTCTGGCTTTTGGTTATGTGGATGATGTGGTATT
S8    AGGGCTTTCCCCCACTGTCTGGCTTTTGGTTATGTGGATGATGTGGTATT
S12   AGGGCTTTCCCCCACTGTCTGGCTTTTGGTTATGTGGATGATGTGGTATT
S15   AGGGCTTTCCCCCACTGTCTGGCTTTTGGTTATGTGGATGATGTGGTATT

          910        920        930        940        950
S0    GGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGNTACCA
S6    GGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTACCA
S8    GGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTACCA
S12   GGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTACCA
S15   GGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTACCA

          960        970        980        990       1000
S0    ATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAACAAAACAAAAAGA
S6    ATTTTCTTTTGTCTTTGGGTATACATTTAAATCCTAACAAAACAAAAAGA
S8    ATTTTCTTTTGTCTTTGGGTATACATTTAAATCCTAACAAAACAAAAAGA
S12   ATTTTCTTTTGTCTTTGGGTATACATTTAAATCCTAACAAAACAAAAAGA
S15   ATTTTCTTTTGTCTTTGGGTATNCATTTAAATCCTAACAAAACAAAAAGA

         1010       1020       1030       1040       1050
```

Figure 7 (continued)

```
S0      TGGGGTTACTCCCTACATTTTATGGGCTATGTCATTGGAT
S6      TGGGGTTACTCCCTACATTTTATGGGCTATGTCATTGGATGTCATGGGTC
S8      TGGGGTTACTCCCTACATTTTATGGGCTATGTCATTGGATGTCATGGGTC
S12     TGGGGTTACTCCCTACATTTTATGGGCTATGTCATTGGATGTCATGGGTC
S15     TGGGGTTACTCCCTACA

              1060       1070       1080       1090       1100
S0
S6      CTTGCCACAAGAACACATCAGACAAAAAATCAAAGAATGTTTTAGAAAAC
S8      CTTGCCACAAGAACACATCAGACAAAAAATCA
S12     CTTGCCACAAGAACACATCAGACAAAAAATCAAAGAATGTTTTAGAAAAC
S15
```

Figure 7 (continued)

Patient B Am

```
                260        270        280        290        300
S0     SGHTTNFASKSTSCLHQSPVRKAAYPAVSTFERHSSSGHAVELNNLPPNS   [SEQ ID NO:25]
S6                                                          [SEQ ID NO:26]
S8                CPFCLHQSPVRKAAYPAVSTFERHSSSGHAVELNNLPPNS  [SEQ ID NO:27]
S12    SGHITNSASKSTSCLHQSPVRKAAYPAVSTFERHSSSGHAVELNNLPPNS   [SEQ ID NO:28]
S15        TNFASKSTSCLHQSPVRKAAYPAVSTFERHSSSGHAVELNNLPPNS   [SEQ ID NO:29]

          310        320        330        340        350
S0     ARSQSERPVFPCWWLQFRNSKPCSDYCLSLIVNLLEDWGPCAEHGEHHIR
S6
S8     ARSQSERPVFPCWWLQFRNSKPCSDYCLSLIVNLLEDWGPCAEHGEHHIR
S12    ARSQSERPVFPCWWLQFRNSKPCSDYCLSLIVNLLEDWGPCAEHGEHHIR
S15    ARSQSERPVFPCWWLQFRNSKPCSDYCLSLIVNLLEDWGPCAEHGEHHIR

          360        370        380        390        400
S0     TPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGDYRVSWPKFAVPN
S6
S8     TPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGDYRVSWPKFAVPN
S12    TPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGDYRVSWPKFAVPN
S15    TPRTPSRVTGGVFXVDKNPHNTAESRLVVDFSQFSRGDYRVSWPKFAVPN

          410        420        430        440        450
S0     LQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARL
S6               SNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARL
S8     LQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARL
S12    LQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARL
S15    LQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSTGLSRYVARL

          460        470        480        490        500
S0     SSNSRILNHQHGTMPNLHDSCSRNLYGSLMLLYQTFGRKLHLYSHPIILG
S6     SSNSRILNHQQGTMPNLHDSCSRNLYGSLMLLYQTFGRKLHLYSHPIILG
S8     SSNSRILNHQQGTMPNLHDSCSRNLYGSLMLLYQTFGRKLHLYSHPIILG
S12    SSNSRILNHQQGTMPNLHDSCSRNLYGSLMLLYQTFGRKLHLYSHPIILG
S15    SSNSRILNHQQGTMPNLHDSCSRNLYGSLMLLYQTFGRKLHLYSHPIILG

          510        520        530        540        550
S0     FRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVS
S6     FRKIPMGVGLSPFLMAQFTSAICSVVRRAFPHCLAFGYVDDVVLGAKSVS
S8     FRKIPMGVGLSPFLMAQFTSAICSVVRRAFPHCLAFGYVDDVVLGAKSVS
S12    FRKIPMGVGLSPFLMAQFTSAICSVVRRAFPHCLAFGYVDDVVLGAKSVS
S15    FRKIPMGVGLSPFLMAQFTSAICSVVRRAFPHCLAFGYVDDVVLGAKSVS

          560        570        580        590        600
S0     HLESLFTAXTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIGCHGSXPQEHI
S6     HLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIG
S8     HLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIGCHGSLPQEHI
S12    HLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLHFMGYVIG
S15    HLESLFTAVTNFLLSLGXHLNPNKTKRWGYSL
```

Figure 8

```
                10        20        30        40        50
S0                                                        [SEQ ID NO:30]
S6                                                        [SEQ ID NO:31]
S8                                                        [SEQ ID NO:32]
S12   LGSPQAQGILQTLPANPPPASTNRQSGRQPTPLSPPLRDTHPQAMQWNST  [SEQ ID NO:33]
S15                     PPPASTNRQSGRQPTPLSPPLRDTHPQAMQWNST  [SEQ ID NO:34]

                60        70        80        90        100
S0
S6
S8
S12   TFHQTLQDPRVKGLYFPAGGSSSGTVNPVPTTASHSSSIFSRIGVPALNM
S15   TFHQTLQDPRVKGLYFPAGGSSSGTVNPVPTTASHSSSIFSRIGVPALNM

                110       120       130       140       150
S0                                 QSLDSWWTSLNFRGGTTVCLGQ
S6
S8
S12   ENITSGLLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFRGGTTVCLGQ
S15   ENITSGLLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFRGGTTVCLGQ

                160       170       180       190       200
S0    NSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDCQ
S6                  PPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDCQ
S8                   PTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDCQ
S12   NSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDCQ
S15   NSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLALLDCQ

                210       220       230       240       250
S0    GMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSTVPSCCCTKPSDGNCTCIP
S6    GMLPVCPLIPGSSTTSRGPCRTCTTPAQGTSTVPSCCCTKPSDGNCTCIP
S8    GMLPVCPLIPGSSTTSRGPCRTCTTPAQGTSTVPSCCCTKPSDGNCTCIP
S12   GMLPVCPLIPGSSTTSRGPCRTCTTPAQGTSTVPSCCCTKPSDGNCTCIP
S15   GMLPVCPLIPGSSTTSRGPCRTCTTPAQGTSTVPSCCCTKPSDGNCTCIP

                260       270       280       290       300
S0    IPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLLVIWMMWYW
S6    IPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLLVMWMMWYW
S8    IPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLLVMWMMWYW
S12   IPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLLVMWMMWYW
S15   IPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLLVMWMMWYW

                310       320
S0    GPSLYRILSPFLPLXPIFFCLWVYI
S6    GPSLYRILSPFLPLLPIFFCLWVYI
S8    GPSLYRILSPFLPLLPIFFCLWVYI
S12   GPSLYRILSPFLPLLPIFFCLWVYI
S15   GPSLYRILSPFLPLLPIFFCLWVXI
```

Figure 9

```
        10         20         30         40         50         60         70         80         90        100
TACTACAAACCTTGCCAGCAAATCCGCCTCCTGCCTCTACCAATCGCCAGTCAGGAAGGCAGCCTACCCCTCTGACTCCACCTTTGAGAAACACTCATCC

       110        120        130        140        150        160        170        180        190        200
TCAGGCCATGCAGTGGAACTCCACAAACTTCCACCGAACTCTACAAGATCCCAGAGTGAAAGGCCTGTATCTCCCTGCTGGTGGCTCCAGTTCAGGAACA

       210        220        230        240        250        260        270        280        290        300
GTAAACCCTGTTCCGACTACTGTCTCTCACACATCGTCAATCTTATCGAGGATTGGGGACCCTGCACTGAACATGGAGAACATCACATCAGGATTCCTAG

       310        320        330        340        350        360        370        380        390        400
GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGG

       410        420        430        440        450        460        470        480        490        500
GGGGACCACCGTGTGCCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGCTGGATGTGT

       510        520        530        540        550        560        570        580        590        600
CTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGCCCGTTTGCCCTCTAA

       610        620        630        640        650        660        670        680        690        700
TTCCAGGATCCTCAACCACCAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTWTGTATCCCTCATGTTGCTGTACCAAACCTWC

       710        720        730        740        750        760        770        780        790        800
GGMCGSAAATTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGACTCAGTTTACTA

       810        820        830        840        850        860        870        880        890        900
GTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAGGTCTGTACAGCATCGTGA

       910        920        930        940        950        960        970        980        990       1000
GGCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTCTGGGTATACATTTAACCCCGGACAAAACAAAAAGATGGGGTTACTCTTTACATTTCATGGGC

      1010       1020       1030
TATGTCATTGGATGTTATGGGTCATTGCCAC
```

Figure 10

```
         10         20         30         40         50         60         70         80         90        100
TTNLASKSASCLYQSPVRKAAYPSDSTFEKHSSSGHAVELHKLPPNSTRSQSERPVSPCWWLQFRNSKPCSDYCLSHIVNLIEDWGPCTEHGEHHIRIPR

        110        120        130        140        150        160        170        180        190        200
TPARVTGGVFLVDKNPHNTAESRLVVDFSQFSRGDHRVPWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLPSN

        210        220        230        240        250        260        270        280        290        300
SRILNHQHGTMQNLHDSCSRNLY/FVSLMLLYQTF/TGRKLHLYSHPIILGFRKIPMGVGLSPFLLTQFTSAICSVVRRAFPHCLAFSYMDDVVLGARSVQ

        310        320        330        340
HREALFTAVTNFLLSLGIHLTPDKTKRWGYSLHFMGYVIGCYGSLP
```

Figure 11

```
         10         20         30         40         50         60         70         80         90         100
LQTLPANPPPASTNRQSGRQPTPLTPPLRNTHPQAMQWNSTNFHRTLQDPRVKGLYLPAGGSSSGTVNPVPTTVSHTSSILSRIGDPALNMENITSGFLG

         110        120        130        140        150        160        170        180        190        200
PLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLI

         210        220        230        240        250        260        270        280        290        300
PGSSTTSTGPCRTCTTPAQGTSM/LYPSCCCTKPS/TAANCTCIPIPSSWAFGKFLWEWASARFS*LSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPGLYS

         310
IVRPFLPLLPIFFCLWVYI
```

Figure 12

```
        10         20         30         40         50         60         70         80         90        100
TGGTCACAGTGCCAACAGTTCCTCCTCCTGCCTCCACCAATCGGCAGTCAGGGAGGCAGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCA

       110        120        130        140        150        160        170        180        190        200
GGCCATGGTGGCTCAGCCTGCTGGTGGCTCCAGTTCAGGAACACTCAACCCTGTTCCCAATATTGCCTCTCACATCTCGTCAATCTCCTTGAGGACTGGG

       210        220        230        240        250        260        270        280        290        300
GACCCTGCGCCGAACATGGAGAACATCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATAC

       310        320        330        340        350        360        370        380        390        400
CGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAGTTTTCTAGGGGGATCACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACC

       410        420        430        440        450        460        470        480        490        500
AACCTCCTGTCCTCCAATTTGACCTGGTTATCGCTGGATATGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCCGCTATGCCTCATCTTCTTATTG

       510        520        530        540        550        560        570        580        590        600
GTTCTTCTGGATTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAACCAGTGCGGGACCCTGCAAAACCTGCACGACTCCTGCTC

       610        620        630        640        650        660        670        680        690        700
AAGGCAACTCTATGTTTCCCTCATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTGGGCTTTCGCAAAATACCT

       710        720        730        740        750        760        770        780        790        800
ATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGATTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCTATA

       810        820        830        840        850        860        870        880
TTGATGATGTGGTACTGGGGGCCAAGTCTGCACAACATCTTGAGTCCCTTTATACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTAT
```

Figure 13

```
        10        20        30        40        50        60        70        80        90       100
GHSANSSSSCLHQSAVREAAYSHLSTSKRQSSSGHGGSACWWLQFRNTQPCSQYCLSHLVNLLEDWGPCAEHGEHHIRIPRTPARVTGGVFLVDKNPHNT

       110       120       130       140       150       160       170       180       190       200
AESRLVVDFSQFSRGITRVSWPKFAVPNLQSLTNLLSSNLTWLSLDMSAAFYHIPLHPAAMPHLLIGSSGLSRYVARLSSNSRIHNNQCGTLQNLHDSCS

       210       220       230       240       250       260       270       280       290
RQLYVSLMLLYKTYGWKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVIRRAFPHCLAFSYIDDVVLGAKSAQHLESLYTAVTNFLLSLG
```

Figure 14

```
      10        20        30        40        50        60        70        80        90       100
VTVPTVPPPASTNRQSGRQPTPISPPLRDSHPQAMVAQPAGGSSSGTLNPVPNIASHISSISLRTGDPAPNMENITSGFLGPLLVLQAGFFLLTRILTIP

     110       120       130       140       150       160       170       180       190       200
QSLDSWWTSLSFLGGSPVCLGQNSQSPTSNHSPTSCPPI*PGYRWICLRRFIIFLFILPLCLIFLLVLLDYQGMLPVCPLIPGSTTTSAGPCKTCTTPAQ

     210       220       230       240       250       260       270       280       290
GNSMFPSCCCTKPTDGNCTCIPIPSSWAPAKYLWEWASVRFSWLSLLVPFVQ*FVGLSPTVWLSAILMWYWGPSLHNILSPFIPLLPIFFCLWV
```

Figure 15

```
        10         20         30         40         50
TCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTT

        60         70         80         90        100
TATGATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGTTC

       110        120        130        140        150
TTCTGGATTATCAAGGTATGTTGCCCGTCTGTCCTCTAATTCCAGGATCA

       160        170        180        190        200
ACAACAACCAGTACGGGACCATGCAAAACCAAAACCTGCACGACTCCTGC

       210        220        230        240        250
TCAAGGCAACTCTATGTTTCCCTCATGTTGCTGTACAAAACCTACGGATG

       260        270        280        290        300
GAAATTGCACCTGTATTCCCATCCCATCGTCCTGGGCTTTCGCAAAATTC

       310        320        330        340        350
CTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACTAGTGCC

       360        370        380        390        400
ATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCTA

       410        420        430        440        450
TATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCGTGAGGCCC

       460        470        480        490        500
TTTATACAGCTGTTACCAATTTTCTTTTGTCTCTGGGTATACATTTAAAC

       510        520        530        540        550
CCTAACAAAACAAAAAGATGGGGTTATTCCCTAAACTTCATGGGTTACAT

       560        570        580        590
AATTGGAAGTTGGGGAACATTGCCACAGGATCATATTGTAC
```

Figure 16

```
         10         20         30         40         50
SNLSWLSLDVSAAFYDIPLHPAAMPHLLIGSSGLSRYVARLSSNSRINNN

         60         70         80         90        100
QYGTMQNQNLHDSCSRQLYVSLMLLYKTYGWKLHLYSHPIVLGFRKIPMG

        110        120        130        140        150
VGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHREALYT

        160        170        180
AVTNFLLSLGIHLNPNKTKRWGYSLNFMGYIIGSWG
```

Figure 17

```
         10         20         30         40         50
SCPPICPGYRWMCLRRFMIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGS

         60         70         80         90        100
TTTSTGPCKTKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAKF

        110        120        130        140        150
LWEWASVRFSWLSLLVPFVQWFVGLSPTVWLSAIWMMWYWGPSLYSIVRP

        160
FIQLLPIFFCLWVYI
```

Figure 18

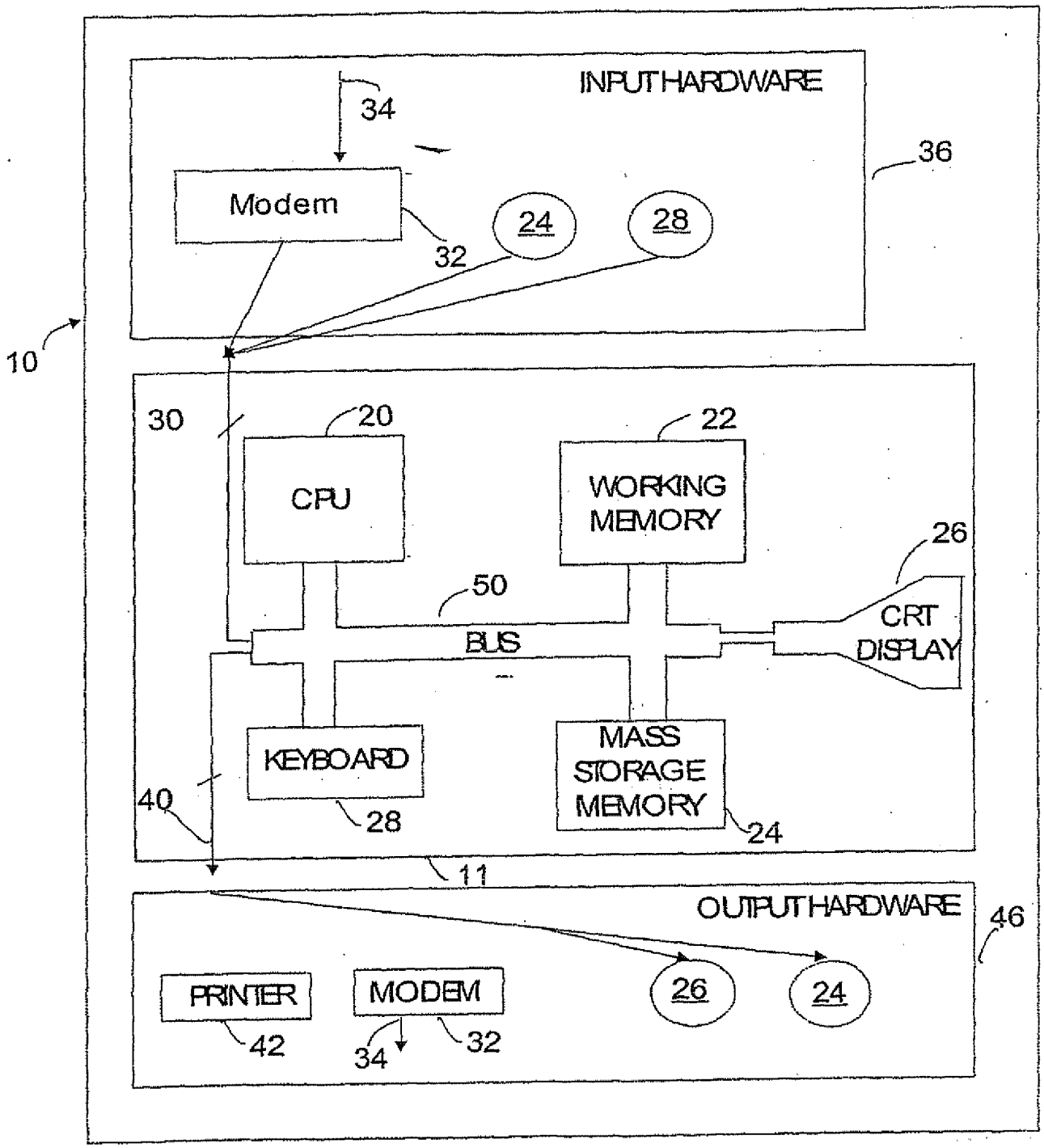
INPUT HARDWARE
34
36
Modem
32
24
28
10
30
20
CPU
22
WORKING MEMORY
26
50
BUS
CRT DISPLAY
KEYBOARD
28
MASS STORAGE MEMORY
24
40
11
OUTPUT HARDWARE
46
PRINTER
42
MODEM
34
32
26
24

Figure 19

```
        10         20         30         40         50
AATCCTCACAATACCGCAGAGTCTAGACTTCGTGGTGACTTCTCTCAATT

        60         70         80         90         100
TTCTAGGGGACCACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCT

        110        120        130        140        150
CCAATCACTCACCAACCTCTTGTCCTCCAATTTGTCCTGGTTATCGCTGG

        160        170        180        190        200
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        210        220        230        240        250
CATCTTCTTATTGGTTCTTCTGGATTATCAAGGTATGTTGCCCGTTTGTC

        260        270        280        290        300
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        310        320        330        340        350
ACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTCATGTTGCTGTACAAA

        360        370        380        390        400
ACCTACGGATGGAAATTGCACMTGTATTCCCATCCCATCATCTTGGGCTT

        410        420        430        440        450
TCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGTTCAGT

        460        470        480        490        500
TTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTG

        510        520        530        540        550
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        560        570        580        590        600
TCTTGAGTCCCTTTATACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTA

        610        620        630        640        650
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ATGGGTTATGTAATTGGAA
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Figure 20

```
         10         20         30         40        50
SNLSWLSLDVSAAFYHIPLHPAAMPHLLIGSSGLSRYVARLSSNSRIHNN

         60         70         80         90        100
QYGTLQNLHDSCSRQLYVSLMLLYKTYGWKLHXYSHPIILGFRKIPMGVG

        110        120        130        140        150
LSPFLLVQFTSAICSVVRRAFPHCLAFSYMDDIVLGAKSVQHLESLYTAV

        160        170        180
TNFLLSLGIHLTPNKTKRWGYSLNFMGYVIG
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Figure 21

```
         10         20         30         40         50
PICPGYRWMCLRRFIISLFILLLCLIFLLVLLDYQGMLPVCPLIPGSTTT

         60         70         80         90        100
STGPCKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAKYLWEWA

        110        120        130        140        150
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PIFFCLWVYI
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Figure 22

```
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         60         70         80         90        100
TCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGAC

        110        120        130        140        150
TATCAAGGTATGTTGCCCGTTTGTCCTCTACTTCCAGGAACATCAACTAC

        160        170        180        190        200
CAGCACGGGACCATGCAAGACCTGCACGACTCCTGCTCAAGGAACCTCTA

        210        220        230        240        250
TGTTTCCCTCTTGTTGCTGTACAAAACCTTCGGACGGAAATTGCACTTGT

        260        270        280        290        300
ATTCCCATCCCATCGTCTTGGGCTTTCGCAAGATTCCTATGGGAGTGGGC

        310        320        330        340        350
CTCAGTCCGTTTCTCTTGGCTCARTTTACTAGTGCCATTTGTTCAGTGGT

        360        370        380        390        400
TCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATTGATGATGTGG

        410        420        430        440        450
TATTGGGGGCCAAGTCTGTACAACATCTTGAATCCCTTTTTACCTCTATT

        460        470        480        490        500
ACCAATTTTCTTATGTCTTTGGGTATACATTTAAACCCTAAGAAAACCAA

        510        520        530        540        550
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Figure 23

```
         10         20         30         40         50
SNLSWVSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSTSRNINY

         60         70         80         90        100
QHGTMQDLHDSCSRNLYVSLLLLYKTFGRKLHLYSHPIVLGFRKIPMGVG

        110        120        130        140        150
LSPFLLAQFTSAICSVVRRAFPHCLAFSYIDDVVLGAKSVQHLESLFTSI

        160        170        180
TNFLMSLGIHLNPKKTKRWGYSLNFMGYVIGSWG
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Figure 24

```
        10         20         30         40         50
RICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGTSTT

        60         70         80         90         100
STGPCKTCTTPAQGTSMFPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWA

        110        120        130        140        150
SVRFSWLXLLVPFVQWFVGLSPTVWLSVILMMWYWGPSLYNILNPFLPLL

        160
PIFLCLWVYI
```

Figure 25

```
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        60         70         80         90        100
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       110        120        130        140        150
GAACTCCACAACCTTCCACCAAACTCTGCWAGATCCCAGAGTGAGAGGCC
       160        170        180        190        200
TGTATTTCCCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCG
       210        220        230        240        250
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       310        320        330        340        350
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       360        370        380        390        400
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       410        420        430        440        450
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       460        470        480        490        500
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       660        670        680        690        700
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       710        720        730        740        750
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       760        770        780        790        800
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       960        970        980        990       1000
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Figure 26

```
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       160        170        180        190        200
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       210        220        230        240        250
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       260        270        280        290        300
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       310        320        330        340
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Figure 27

```
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        110        120        130        140        150
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        160        170        180        190        200
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        210        220        230        240        250
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Figure 28

SEQUENCE LISTING

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Southern Health (for all States except the US)
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Locarnini, Stephen (US only)
Ayres, Anna (US only)
Colledge, Danielle (US only)
Sasadeusz, Joseph (US only)
Tillmann, Hans (US only)
Angus, Peter (US only)
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Lys Leu Ala Ser Lys Ser Ala Ser Ser Ile Xaa Gln Ser Pro Val Arg
1 5 10 15

Xaa Ala Ala Tyr Pro Ala Val Ser Thr Phe Glu Lys His Ser Ser Ser
20 25 30

Gly His Ala Val Glu Xaa His Asn Leu Pro Pro Asn Ser Xaa Arg Ser
35 40 45

Gln Xaa Glu Arg Pro Val Phe Pro Cys Trp Trp Leu Gln Phe Arg Asn
50 55 60

Ser Lys Pro Cys Ser Asp Tyr Cys Leu Ser His Ile Val Asn Leu Leu
65 70 75 80

Glu Asp Trp Gly Pro Cys Ala Glu His Gly Glu His His Ile Arg Ile
85 90 95

Pro Arg Thr Pro Xaa Arg Val Thr Gly Gly Val Phe Leu Val Asp Lys
100 105 110

Asn Pro His Asn Thr Ala Glu Ser Arg Leu Val Val Asp Phe Ser Gln
115 120 125

Phe Ser Arg Gly Asn Tyr Arg Val Ser Trp Pro Lys Phe Ala Val Pro
130 135 140

Asn Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu
145 150 155 160

Ser Leu Asp Val Ser Ala Ala Phe Tyr His Leu Pro Leu His Pro Ala
165 170 175

Ala Met Pro His Leu Leu Val Gly Ser Ser Gly Leu Ser Arg Tyr Val
180 185 190

Ala Arg Leu Ser Ser Asn Ser Arg Ile Phe Asn His Gln Arg Gly Xaa
195 200 205

Met Gln Asn Leu His Asp Tyr Cys Ser Arg Asn Leu Tyr Val Ser Leu
210 215 220

Leu Leu Leu Tyr Gln Thr Phe Gly Arg Lys Leu His Leu Tyr Ser His
225 230 235 240

Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro Met Gly Val Gly Leu Ser
245 250 255

Pro Phe Leu Leu Ala Gln Phe Thr Ser Ala Ile Cys Ser Val Val Arg
260 265 270

Arg Ala Phe Pro His Cys Leu Ala Phe Ser Tyr Met Asp Asp Val Val
275 280 285

Leu Gly Ala Lys Ser Val Xaa His Leu Glu Ser Leu Phe Thr Ala Val
290 295 300

Thr Asn Phe Leu Leu Ser Leu Gly Ile His Leu Asn Pro Asn Lys Thr
305 310 315 320

Lys Arg Trp Gly Tyr Ser Leu His Phe Met Gly Tyr Val Ile Gly Cys
325 330 335

<210> 20
<211> 340
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans 2

<400> 20

His Thr Thr Asn Phe Ala Ser Lys Ser Ala Ser Cys Leu His Gln Ser
1 5 10 15

Pro Val Arg Lys Ala Ala Tyr Pro Ala Val Ser Thr Phe Glu Lys His
20 25 30

Ser Ser Ser Gly His Ala Val Glu Phe His Asn Leu Pro Pro Asn Ser
35 40 45

Ala Arg Ser Gln Ser Glu Arg Pro Val Phe Pro Cys Trp Trp Leu Gln
50 55 60

Phe Arg Asn Ser Lys Pro Cys Ser Asp Tyr Cys Leu Ser Leu Ile Val
65 70 75 80

Asn Leu Leu Glu Asp Trp Gly Pro Cys Ala Glu His Gly Glu His His
85 90 95

Ile Arg Ile Pro Arg Thr Pro Ser Arg Val Thr Gly Gly Val Phe Leu
100 105 110

Val Asp Lys Asn Pro His Asn Thr Ala Glu Ser Arg Leu Val Val Asp
115 120 125

Phe Ser Gln Phe Ser Arg Gly Asn Tyr Arg Val Ser Trp Pro Lys Phe
130 135 140

Ala Val Pro Asn Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu
145 150 155 160

Ser Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His Leu Pro Leu
165 170 175

His Pro Ala Ala Met Pro His Leu Leu Val Gly Ser Ser Gly Leu Ser
180 185 190

Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile Leu Asn Asn Gln
195 200 205

His Gly Thr Met Pro Asp Leu His Asp Tyr Cys Ser Arg Asn Leu Tyr
210 215 220

Val Ser Leu Leu Leu Leu Tyr Gln Thr Phe Gly Arg Lys Leu His Leu
225 230 235 240

Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro Met Gly Val
245 250 255

Gly Leu Ser Pro Phe Leu Leu Ala Gln Phe Thr Ser Ala Ile Cys Ser
260 265 270

Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe Ser Tyr Met Asp
275 280 285

Asp Val Val Leu Gly Ala Lys Ser Val Gln His Leu Glu Ser Leu Phe
290 295 300

Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly Ile His Leu Asn Pro
305 310 315 320

Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu Asn Phe Met Gly Tyr Val
325 330 335

Ile Gly Cys Tyr
340

<210> 21
<211> 344
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans 3

<400> 21

Leu Ala Gln Gly Ile Leu Gln Asn Phe Ala Ser Lys Ser Ala Ser Cys
1 5 10 15

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Leu His Gln Ser Pro Val Arg Lys Ala Ala Tyr Pro Ala Val Ser Thr
            20                  25                  30

Phe Glu Lys His Ser Ser Ser Gly His Ala Val Glu Phe His Asn Leu
        35                  40                  45

Pro Pro Asn Ser Ala Arg Ser Gln Ser Glu Arg Pro Val Phe Pro Cys
    50                  55                  60

Trp Trp Leu Gln Phe Arg Asn Ser Lys Pro Cys Ser Asp Tyr Cys Leu
65                  70                  75                  80

Ser Leu Ile Val Asn Leu Leu Glu Asp Trp Gly Pro Cys Ala Glu His
                85                  90                  95

Gly Glu His His Ile Arg Ile Pro Arg Thr Pro Ser Arg Val Thr Gly
            100                 105                 110

Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala Glu Ser Arg
        115                 120                 125

Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Tyr Arg Val Ser
    130                 135                 140

Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr Asn Leu Leu
145                 150                 155                 160

Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr
                165                 170                 175

His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu Val Gly Ser
            180                 185                 190

Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile
        195                 200                 205

Leu Asn Asn Gln His Gly Thr Met Pro Asp Leu His Asp Tyr Cys Ser
    210                 215                 220

Arg Asn Leu Tyr Val Ser Leu Leu Leu Leu Tyr Gln Thr Phe Gly Arg
225                 230                 235                 240
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Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile
245 250 255

Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln Phe Thr Ser
260 265 270

Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe
275 280 285

Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val Gln His Leu
290 295 300

Glu Ser Leu Phe Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly Ile
305 310 315 320

His Leu Asn Pro Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu Asn Phe
325 330 335

Met Gly Tyr Val Ile Gly Cys Tyr
340

<210> 22
<211> 336
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans 4

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> x =any amino acid

<400> 22

Ala Ser Lys Ser Ala Ser Ser Ile Tyr Gln Ser Pro Val Gly Thr Ala
1 5 10 15

Ala Tyr Pro Ala Val Ser Thr Xaa Glu Lys His Ser Ser Ser Gly His
20 25 30

Ala Val Glu Leu His Asn Leu Pro Pro Asn Ser Glu Arg Ser Gln Gly
35 40 45

```
Glu Arg Pro Val Phe Pro Cys Trp Trp Leu Gln Phe Arg Asn Ser Lys
    50                  55                  60

Pro Cys Ser Asp Tyr Cys Leu Ser His Ile Val Asn Leu Leu Glu Asp
65                  70                  75                  80

Trp Gly Pro Cys Ala Glu His Gly Glu His His Ile Arg Ile Pro Arg
                85                  90                  95

Thr Pro Ala Arg Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro
            100                 105                 110

His Asn Thr Ala Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser
        115                 120                 125

Arg Gly Asn Tyr Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu
    130                 135                 140

Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu
145                 150                 155                 160

Asp Val Ser Ala Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met
                165                 170                 175

Pro His Leu Leu Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg
            180                 185                 190

Leu Ser Ser Asn Ser Arg Ile Phe Asn His Gln Arg Gly Asn Met Gln
        195                 200                 205

Asn Leu His Asp Cys Cys Ser Arg Asn Leu Tyr Val Ser Leu Leu Leu
    210                 215                 220

Leu Tyr Gln Thr Phe Gly Arg Lys Leu His Leu Tyr Ser His Pro Ile
225                 230                 235                 240

Ile Leu Gly Phe Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe
                245                 250                 255

Leu Leu Ala Gln Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala
            260                 265                 270

Phe Pro His Cys Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly
```

275 280 285

Ala Lys Ser Val Gln His Leu Glu Ser Leu Phe Thr Ala Val Thr Asn
290 295 300

Phe Leu Leu Ser Leu Gly Ile His Leu Asn Pro Asn Lys Thr Lys Arg
305 310 315 320

Trp Gly Tyr Ser Leu Asn Phe Met Gly Tyr Val Ile Gly Trp Tyr Gly
325 330 335

<210> 23
<211> 226
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Pre 1

<220>
<221> MISC_FEATURE
<222> (120)..(120)
<223> x = any amino acid

<220>
<221> MISC_FEATURE
<222> (208)..(208)
<223> x = any amino acid

<400> 23

Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln
1 5 10 15

Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu
20 25 30

Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys
35 40 45

Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser
50 55 60

Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe
65 70 75 80

Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val
85 90 95

Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly
100 105 110

Ser Ser Thr Thr Ser Ala Gly Xaa Cys Arg Thr Cys Thr Thr Thr Ala
115 120 125

Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp
130 135 140

Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys
145 150 155 160

Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu
165 170 175

Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu
180 185 190

Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Xaa
195 200 205

Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val
210 215 220

Tyr Ile
225

<210> 24
<211> 309
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of 2

<400> 24

Pro Pro Pro Ala Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro
1 5 10 15

Leu Ser Pro Pro Leu Arg Asn Thr His Pro Gln Ala Met Gln Trp Asn
20 25 30

Ser Thr Thr Phe His Gln Thr Leu Gln Asp Pro Arg Val Arg Gly Leu
35 40 45

Tyr Phe Pro Ala Gly Gly Ser Ser Ser Gly Thr Val Asn Pro Val Leu
50 55 60

Thr Thr Ala Ser Pro Leu Ser Ser Ile Phe Ser Arg Ile Gly Asp Pro
65 70 75 80

Ala Leu Asn Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu
85 90 95

Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
100 105 110

Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr
115 120 125

Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser
130 135 140

Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu
145 150 155 160

Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe
165 170 175

Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu
180 185 190

Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met
195 200 205

Thr Thr Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys
210 215 220

Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
225 230 235 240

Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu
245 250 255

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
260 265 270

Val Trp Leu Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
275 280 285

Tyr Ser Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys
290 295 300

Leu Trp Val Tyr Ile
305

<210> 25
<211> 309
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of 3

<400> 25

Pro Pro Pro Ala Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro
1 5 10 15

Leu Ser Pro Pro Leu Arg Asn Thr His Pro Gln Ala Met Gln Trp Asn
20 25 30

Ser Thr Thr Phe His Gln Thr Leu Gln Asp Pro Arg Val Arg Gly Leu
35 40 45

Tyr Phe Pro Ala Gly Gly Ser Ser Ser Gly Thr Val Asn Pro Val Leu
50 55 60

Thr Thr Ala Ser Pro Leu Ser Ser Ile Phe Ser Arg Ile Gly Asp Pro
65 70 75 80

Ala Leu Asn Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu
85 90 95

Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
100 105 110

Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr
115 120 125

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Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser
    130                 135                 140

Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu
145                 150                 155                 160

Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe
                165                 170                 175

Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu
            180                 185                 190

Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met
        195                 200                 205

Thr Thr Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys
    210                 215                 220

Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
225                 230                 235                 240

Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu
                245                 250                 255

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
            260                 265                 270

Val Trp Leu Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
        275                 280                 285

Tyr Ser Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys
    290                 295                 300

Leu Trp Val Tyr Ile
305
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<210> 26
<211> 309
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of 4

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> x = any amino acid

<220>
<221> MISC_FEATURE
<222> (46)..(46)
<223> x = any amino acid

<400> 26

Pro Pro Pro Pro Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro
1 5 10 15

Leu Ser Pro Pro Xaa Arg Asn Thr His Pro Gln Ala Met Gln Trp Asn
20 25 30

Ser Thr Thr Phe His Gln Thr Leu Lys Asp Pro Arg Val Xaa Gly Leu
35 40 45

Tyr Phe Pro Ala Gly Gly Ser Ser Ser Gly Thr Val Asn Pro Val Pro
50 55 60

Thr Thr Ala Ser Pro Ile Ser Ser Ile Phe Ser Arg Ile Gly Asp Pro
65 70 75 80

Ala Leu Asn Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu
85 90 95

Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
100 105 110

Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr
115 120 125

Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser
130 135 140

Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu
145 150 155 160

Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe
165 170 175

Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu
180 185 190

Ile Pro Gly Ser Ser Thr Thr Ser Ala Gly Thr Cys Arg Thr Cys Thr
195 200 205

Thr Ala Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys
210 215 220

Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
225 230 235 240

Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu
245 250 255

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
260 265 270

Val Trp Leu Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
275 280 285

Tyr Ser Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys
290 295 300

Leu Trp Ala Tyr Ile
305

<210> 27
<211> 656
<212> DNA
<213> artificial sequence

<220>
<223> SO

<220>
<221> misc_feature
<222> (561)..(561)
<223> n = any nucleotide

<400> 27
cgcagagtct agactcgtgg tggacttctc tcaattttcg agggggggact accgtgtgtc 60

ttggccaaaa ttcgcagtcc ccaacctcca atcactcacc aacctcctgt cctccaactt 120

gtcctggtta tcgctggatg tgtctgcggc gttttatcat cttcctcttc atcctgctgc 180

tatgcctcat cttcttgttg gttcttctgg actgtcaagg tatgttgccc gtttgtcctc 240

taattccagg atcctcaacc acgagcacgg gaccatgccg aacctgcacg actcctgctc 300

aaggaacctc tacggttccc tcatgttgct gtaccaaacc ttcggacgga aattgcacct 360

gtattcccat cccatcatcc tgggctttcg gaaaattcct atgggagtgg gcctcagccc 420

gtttctcctg gctcagttta ctagtgccat ttgttcagtg gttcgtaggg ctttccccca 480

ctgtctggct tttagttata tggatgatgt ggtattgggg gccaagtctg tatcgcatct 540

tgagtccctt tttaccgctg ntaccaattt tcttttgtct ttgggtatac atttaaaccc 600

taacaaaaca aaaagatggg gttactccct acattttatg ggctatgtca ttggat 656

<210> 28
<211> 625
<212> DNA
<213> artificial sequence

<220>
<223> S6

<220>
<221> misc_feature
<222> (10)..(10)
<223> n = any nucleotide

<400> 28
ttactcaccn acctcctgtc ctccaacttg tcctggttat cgctggatgt gtctgcggcg 60

ttttatcatc ttcctcttca tcctgctgct atgcctcatc ttcttgttgg ttcttctgga 120

ctgtcaaggt atgttgcccg tttgtcctct aattccagga tcctcaacca ccagcagggg 180

accatgccga acctgcacga ctcctgctca aggaacctct acggttccct catgttgctg 240

taccaaacct tcggacggaa attgcacctg tattcccatc ccatcatcct gggctttcgg 300

aaaattccta tgggagtggg cctcagcccg tttctcatgg ctcagtttac tagtgccatt 360

tgttcagtgg ttcgtagggc tttcccccac tgtctggctt ttggttatgt ggatgatgtg 420

gtattggggg ccaagtctgt atcgcatctt gagtcccttt ttaccgctgt taccaatttt 480

cttttgtctt tgggtataca tttaaatcct aacaaaacaa aaagatgggg ttactcccta 540

cattttatgg gctatgtcat tggatgtcat gggtccttgc cacaagaaca catcagacaa 600

aaaatcaaag aatgttttag aaaac 625

<210> 29

<211> 1033
<212> DNA
<213> artificial sequence

<220>
<223> S8

<400> 29
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tttgagagac actcatcctc aggccatgca gtggaactca acaaccttcc accaaactct 120

gcaagatccc agagtgaaag gcctgtattt ccctgctggt ggctccagtt caggaacagt 180

aaaccctgtt ccgactactg cctctcactc atcgtcaatc ttctcgagga ttggggtccc 240

tgcgctgaac atggagaaca tcacatcagg actcctagga ccccttctcg tgttacaggc 300

ggggtttttc ttgttgacaa gaatcctcac aataccgcag agtctagact cgtggtggac 360

ttctctcaat tttcgagggg ggactaccgt gtgtcttggc caaaattcgc agtccccaac 420

ctccaatcac tcaccaacct cctgtcctcc aacttgtcct ggttatcgct ggatgtgtct 480

gcggcgtttt atcatcttcc tcttcatcct gctgctatgc ctcatcttct tgttggttct 540

tctggactgt caaggtatgt tgcccgtttg tcctctaatt ccaggatcct caaccaccag 600

caggggacca tgccgaacct gcacgactcc tgctcaagga acctctacgg ttcccctcatg 660

ttgctgtacc aaaccttcgg acggaaattg cacctgtatt cccatcccat catcctgggc 720

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gccatttgtt cagtggttcg tagggctttc ccccactgtc tggcttttgg ttatgtggat 840

gatgtggtat tgggggccaa gtctgtatcg catcttgagt ccctttttac cgctgttacc 900

aattttcttt tgtctttggg tatacattta aatcctaaca aaacaaaaag atggggttac 960

tccctacatt ttatgggcta tgtcattgga tgtcatgggt ccttgccaca agaacacatc 1020

agacaaaaaa tca 1033

<210> 30
<211> 1100
<212> DNA
<213> artificial sequence

<220>
<223> S12

<400> 30
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cctccaccaa tcgccagtca ggaaggcagc ctaccccgct gtctccacct ttgagagaca 120

ctcatcctca ggccatgcag tggaactcaa caaccttcca ccaaactctg caagatccca 180

gagtgaaagg cctgtatttc cctgctggtg gctccagttc aggaacagta aaaccctgttc 240

cgactactgc ctctcactca tcgtcaatct tctcgaggat tggggtccct gcgctgaaca 300

tggagaacat cacatcagga ctcctaggac cccttctcgt gttacaggcg gggtttttct 360

tgttgacaag aatcctcaca ataccgcaga gtctagactc gtggtggact tctctcaatt 420

ttcgaggggg gactaccgtg tgtcttggcc aaaattcgca gtccccaacc tccaatcact 480

caccaacctc ctgtcctcca acttgtcctg gttatcgctg gatgtgtctg cggcgtttta 540

tcatcttcct cttcatcctg ctgctatgcc tcatcttctt gttggttctt ctggactgtc 600

aaggtatgtt gcccgtttgt cctctaattc caggatcctc aaccaccagc aggggaccat 660

gccgaacctg cacgactcct gctcaaggaa cctctacggt tccctcatgt tgctgtacca 720

aaccttcgga cggaaattgc acctgtattc ccatcccatc atcctgggct ttcggaaaat 780

tcctatggga gtgggcctca gcccgtttct catggctcag tttactagtg ccatttgttc 840

agtggttcgt agggctttcc cccactgtct ggcttttggt tatgtggatg atgtggtatt 900

gggggccaag tctgtatcgc atcttgagtc ccttttacc gctgttacca attttctttt 960

gtctttgggt atacatttaa atcctaacaa aacaaaaaga tggggttact ccctacattt 1020

tatgggctat gtcattggat gtcatgggtc cttgccacaa gaacacatca gacaaaaaat 1080

caaagaatgt tttagaaaac 1100

<210> 31
<211> 987
<212> DNA
<213> artificial sequence

<220>
<223> S15

<220>
<221> misc_feature
<222> (329)..(329)
<223> y = any nucleotide

<220>
<221> misc_feature
<222> (943)..(943)
<223> y = any nucleotide

<400> 31

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ctaccccgct gtctccacct ttgagagaca ctcatcctca ggccatgcag tggaactcaa 120

caaccttcca ccaaactctg caagatccca gagtgaaagg cctgtatttc cctgctggtg 180

gctccagttc aggaacagta aaccctgttc cgactactgc ctctcactca tcgtcaatct 240

tctcgaggat tggggtccct gcgctgaaca tggagaacat cacatcagga ctcctaggac 300

ccttctcgt gttacaggcg gggtttttyt tgttgacaag aatcctcaca ataccgcaga 360

gtctagactc gtggtggact tctctcaatt ttcgaggggg gactaccgtg tgtcttggcc 420

aaaattcgca gtccccaacc tccaatcact caccaacctc ctgtcctcca acttgtcctg 480

gttatcgctg gatgtgtctg cggcgtttta tcatcttcct cttcatcctg ctgctatgcc 540

tcatcttctt gttggctcta ctggactgtc aaggtatgtt gcccgtttgt cctctaattc 600

caggatcctc aaccaccagc aggggaccat gccgaacctg cacgactcct gctcaaggaa 660

cctctacggt tccctcatgt tgctgtacca aaccttcgga cggaaattgc acctgtattc 720

ccatcccatc atcctgggct ttcggaaaat tcctatggga gtgggcctca gcccgtttct 780

catggctcag tttactagtg ccatttgttc agtggttcgt agggctttcc cccactgtct 840

ggcttttggt tatgtggatg atgtggtatt gggggccaag tctgtatcgc atcttgagtc 900

ccttttacc gctgttacca attttctttt gtctttgggt atrcatttaa atcctaacaa 960

aacaaaaaga tggggttact ccctaca 987

<210> 32
<211> 350
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans S0

<220>
<221> MISC_FEATURE
<222> (309)..(309)
<223> x = any amino acid

<220>
<221> MISC_FEATURE
<222> (345)..(345)
<223> x = any amino acid

<400> 32

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Ser Gly His Thr Thr Asn Phe Ala Ser Lys Ser Thr Ser Cys Leu His
1               5                  10                  15

Gln Ser Pro Val Arg Lys Ala Ala Tyr Pro Ala Val Ser Thr Phe Glu
            20                  25                  30

Arg His Ser Ser Ser Gly His Ala Val Glu Leu Asn Asn Leu Pro Pro
        35                  40                  45

Asn Ser Ala Arg Ser Gln Ser Glu Arg Pro Val Phe Pro Cys Trp Trp
    50                  55                  60

Leu Gln Phe Arg Asn Ser Lys Pro Cys Ser Asp Tyr Cys Leu Ser Leu
65                  70                  75                  80

Ile Val Asn Leu Leu Glu Asp Trp Gly Pro Cys Ala Glu His Gly Glu
                85                  90                  95

His His Ile Arg Thr Pro Arg Thr Pro Ser Arg Val Thr Gly Gly Val
            100                 105                 110

Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala Glu Ser Arg Leu Val
        115                 120                 125

Val Asp Phe Ser Gln Phe Ser Arg Gly Asp Tyr Arg Val Ser Trp Pro
    130                 135                 140

Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser
145                 150                 155                 160

Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His Leu
                165                 170                 175

Pro Leu His Pro Ala Ala Met Pro His Leu Leu Val Gly Ser Ser Gly
            180                 185                 190

Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile Leu Asn
        195                 200                 205

His Gln His Gly Thr Met Pro Asn Leu His Asp Ser Cys Ser Arg Asn
    210                 215                 220

Leu Tyr Gly Ser Leu Met Leu Leu Tyr Gln Thr Phe Gly Arg Lys Leu
```

225 230 235 240

His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro Met
245 250 255

Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln Phe Thr Ser Ala Ile
260 265 270

Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe Ser Tyr
275 280 285

Met Asp Asp Val Val Leu Gly Ala Lys Ser Val Ser His Leu Glu Ser
290 295 300

Leu Phe Thr Ala Xaa Thr Asn Phe Leu Leu Ser Leu Gly Ile His Leu
305 310 315 320

Asn Pro Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu His Phe Met Gly
325 330 335

Tyr Val Ile Gly Cys His Gly Ser Xaa Pro Gln Glu His Ile
340 345 350

<210> 33
<211> 181
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans S6

<400> 33

Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His
1 5 10 15

Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu Val Gly Ser Ser
20 25 30

Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile Leu
35 40 45

Asn His Gln Gln Gly Thr Met Pro Asn Leu His Asp Ser Cys Ser Arg
50 55 60

Asn Leu Tyr Gly Ser Leu Met Leu Leu Tyr Gln Thr Phe Gly Arg Lys
65 70 75 80

Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro
85 90 95

Met Gly Val Gly Leu Ser Pro Phe Leu Met Ala Gln Phe Thr Ser Ala
100 105 110

Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe Gly
115 120 125

Tyr Val Asp Asp Val Val Leu Gly Ala Lys Ser Val Ser His Leu Glu
130 135 140

Ser Leu Phe Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly Ile His
145 150 155 160

Leu Asn Pro Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu His Phe Met
165 170 175

Gly Tyr Val Ile Gly
180

<210> 34
<211> 340
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans S8

<400> 34

Cys Pro Phe Cys Leu His Gln Ser Pro Val Arg Lys Ala Ala Tyr Pro
1 5 10 15

Ala Val Ser Thr Phe Glu Arg His Ser Ser Ser Gly His Ala Val Glu
20 25 30

Leu Asn Asn Leu Pro Pro Asn Ser Ala Arg Ser Gln Ser Glu Arg Pro
35 40 45

Val Phe Pro Cys Trp Trp Leu Gln Phe Arg Asn Ser Lys Pro Cys Ser
50 55 60

```
Asp Tyr Cys Leu Ser Leu Ile Val Asn Leu Leu Glu Asp Trp Gly Pro
65                  70                  75                  80

Cys Ala Glu His Gly Glu His His Ile Arg Thr Pro Arg Thr Pro Ser
                85                  90                  95

Arg Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr
            100                 105                 110

Ala Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asp
        115                 120                 125

Tyr Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu
    130                 135                 140

Thr Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser
145                 150                 155                 160

Ala Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu
                165                 170                 175

Leu Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser
            180                 185                 190

Asn Ser Arg Ile Leu Asn His Gln Gln Gly Thr Met Pro Asn Leu His
        195                 200                 205

Asp Ser Cys Ser Arg Asn Leu Tyr Gly Ser Leu Met Leu Leu Tyr Gln
    210                 215                 220

Thr Phe Gly Arg Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly
225                 230                 235                 240

Phe Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Met Ala
                245                 250                 255

Gln Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His
            260                 265                 270

Cys Leu Ala Phe Gly Tyr Val Asp Asp Val Val Leu Gly Ala Lys Ser
        275                 280                 285
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Val Ser His Leu Glu Ser Leu Phe Thr Ala Val Thr Asn Phe Leu Leu
290 295 300

Ser Leu Gly Ile His Leu Asn Pro Asn Lys Thr Lys Arg Trp Gly Tyr
305 310 315 320

Ser Leu His Phe Met Gly Tyr Val Ile Gly Cys His Gly Ser Leu Pro
325 330 335

Gln Glu His Ile
340

<210> 35
<211> 340
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans S12

<400> 35

Ser Gly His Ile Thr Asn Ser Ala Ser Lys Ser Thr Ser Cys Leu His
1 5 10 15

Gln Ser Pro Val Arg Lys Ala Ala Tyr Pro Ala Val Ser Thr Phe Glu
20 25 30

Arg His Ser Ser Ser Gly His Ala Val Glu Leu Asn Asn Leu Pro Pro
35 40 45

Asn Ser Ala Arg Ser Gln Ser Glu Arg Pro Val Phe Pro Cys Trp Trp
50 55 60

Leu Gln Phe Arg Asn Ser Lys Pro Cys Ser Asp Tyr Cys Leu Ser Leu
65 70 75 80

Ile Val Asn Leu Leu Glu Asp Trp Gly Pro Cys Ala Glu His Gly Glu
85 90 95

His His Ile Arg Thr Pro Arg Thr Pro Ser Arg Val Thr Gly Gly Val
100 105 110

Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala Glu Ser Arg Leu Val
115 120 125

Val Asp Phe Ser Gln Phe Ser Arg Gly Asp Tyr Arg Val Ser Trp Pro
130 135 140

Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser
145 150 155 160

Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His Leu
165 170 175

Pro Leu His Pro Ala Ala Met Pro His Leu Leu Val Gly Ser Ser Gly
180 185 190

Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile Leu Asn
195 200 205

His Gln Gln Gly Thr Met Pro Asn Leu His Asp Ser Cys Ser Arg Asn
210 215 220

Leu Tyr Gly Ser Leu Met Leu Leu Tyr Gln Thr Phe Gly Arg Lys Leu
225 230 235 240

His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro Met
245 250 255

Gly Val Gly Leu Ser Pro Phe Leu Met Ala Gln Phe Thr Ser Ala Ile
260 265 270

Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe Gly Tyr
275 280 285

Val Asp Asp Val Val Leu Gly Ala Lys Ser Val Ser His Leu Glu Ser
290 295 300

Leu Phe Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly Ile His Leu
305 310 315 320

Asn Pro Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu His Phe Met Gly
325 330 335

Tyr Val Ile Gly
340

<210> 36
<211> 328
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans S15

<220>
<221> MISC_FEATURE
<222> (110)..(110)
<223> x = any amino acid

<220>
<221> MISC_FEATURE
<222> (314)..(314)
<223> x = any amino acid

<400> 36

Thr Asn Phe Ala Ser Lys Ser Thr Ser Cys Leu His Gln Ser Pro Val
1 5 10 15

Arg Lys Ala Ala Tyr Pro Ala Val Ser Thr Phe Glu Arg His Ser Ser
20 25 30

Ser Gly His Ala Val Glu Leu Asn Asn Leu Pro Pro Asn Ser Ala Arg
35 40 45

Ser Gln Ser Glu Arg Pro Val Phe Pro Cys Trp Trp Leu Gln Phe Arg
50 55 60

Asn Ser Lys Pro Cys Ser Asp Tyr Cys Leu Ser Leu Ile Val Asn Leu
65 70 75 80

Leu Glu Asp Trp Gly Pro Cys Ala Glu His Gly Glu His His Ile Arg
85 90 95

Thr Pro Arg Thr Pro Ser Arg Val Thr Gly Gly Val Phe Xaa Val Asp
100 105 110

Lys Asn Pro His Asn Thr Ala Glu Ser Arg Leu Val Val Asp Phe Ser
115 120 125

Gln Phe Ser Arg Gly Asp Tyr Arg Val Ser Trp Pro Lys Phe Ala Val
130 135 140

```
Pro Asn Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Ser Trp
145                 150                 155                 160

Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His Leu Pro Leu His Pro
                165                 170                 175

Ala Ala Met Pro His Leu Leu Val Gly Ser Thr Gly Leu Ser Arg Tyr
            180                 185                 190

Val Ala Arg Leu Ser Ser Asn Ser Arg Ile Leu Asn His Gln Gln Gly
        195                 200                 205

Thr Met Pro Asn Leu His Asp Ser Cys Ser Arg Asn Leu Tyr Gly Ser
    210                 215                 220

Leu Met Leu Leu Tyr Gln Thr Phe Gly Arg Lys Leu His Leu Tyr Ser
225                 230                 235                 240

His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro Met Gly Val Gly Leu
                245                 250                 255

Ser Pro Phe Leu Met Ala Gln Phe Thr Ser Ala Ile Cys Ser Val Val
            260                 265                 270

Arg Arg Ala Phe Pro His Cys Leu Ala Phe Gly Tyr Val Asp Asp Val
        275                 280                 285

Val Leu Gly Ala Lys Ser Val Ser His Leu Glu Ser Leu Phe Thr Ala
    290                 295                 300

Val Thr Asn Phe Leu Leu Ser Leu Gly Xaa His Leu Asn Pro Asn Lys
305                 310                 315                 320

Thr Lys Arg Trp Gly Tyr Ser Leu
                325
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<210> 37
<211> 197
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of S0

<220>
<221> MISC_FEATURE
<222> (187)..(187)
<223> x = any amino acid

<400> 37

Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Arg Gly Gly Thr
1 5 10 15

Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser
20 25 30

Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu
35 40 45

Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe
50 55 60

Leu Leu Val Leu Leu Asp Cys Gln Gly Met Leu Pro Val Cys Pro Leu
65 70 75 80

Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Thr
85 90 95

Thr Pro Ala Gln Gly Thr Ser Thr Val Pro Ser Cys Cys Cys Thr Lys
100 105 110

Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
115 120 125

Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu
130 135 140

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
145 150 155 160

Val Trp Leu Leu Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
165 170 175

Tyr Arg Ile Leu Ser Pro Phe Leu Pro Leu Xaa Pro Ile Phe Phe Cys
180 185 190

Leu Trp Val Tyr Ile

195

<210> 38
<211> 161
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of S6

<400> 38

Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile
1 5 10 15

Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu
20 25 30

Leu Asp Cys Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser
35 40 45

Ser Thr Thr Ser Arg Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln
50 55 60

Gly Thr Ser Thr Val Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly
65 70 75 80

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe
85 90 95

Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val
100 105 110

Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Leu
115 120 125

Val Met Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Arg Ile Leu
130 135 140

Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr
145 150 155 160

Ile

<210> 39
<211> 160
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of S8

<400> 39

Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
1 5 10 15

Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
20 25 30

Asp Cys Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser
35 40 45

Thr Thr Ser Arg Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln Gly
50 55 60

Thr Ser Thr Val Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn
65 70 75 80

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu
85 90 95

Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
100 105 110

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Leu Val
115 120 125

Met Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Arg Ile Leu Ser
130 135 140

Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
145 150 155 160

<210> 40
<211> 325
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of S12

<400> 40

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Leu Gly Ser Pro Gln Ala Gln Gly Ile Leu Gln Thr Leu Pro Ala Asn
1               5                  10                  15

Pro Pro Pro Ala Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro
            20                  25                  30

Leu Ser Pro Pro Leu Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn
        35                  40                  45

Ser Thr Thr Phe His Gln Thr Leu Gln Asp Pro Arg Val Lys Gly Leu
    50                  55                  60

Tyr Phe Pro Ala Gly Gly Ser Ser Ser Gly Thr Val Asn Pro Val Pro
65                  70                  75                  80

Thr Thr Ala Ser His Ser Ser Ser Ile Phe Ser Arg Ile Gly Val Pro
                85                  90                  95

Ala Leu Asn Met Glu Asn Ile Thr Ser Gly Leu Leu Gly Pro Leu Leu
            100                 105                 110

Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
        115                 120                 125

Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Arg Gly Gly Thr
    130                 135                 140

Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser
145                 150                 155                 160

Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu
                165                 170                 175

Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe
            180                 185                 190

Leu Leu Val Leu Leu Asp Cys Gln Gly Met Leu Pro Val Cys Pro Leu
        195                 200                 205

Ile Pro Gly Ser Ser Thr Thr Ser Arg Gly Pro Cys Arg Thr Cys Thr
    210                 215                 220
```

Thr Pro Ala Gln Gly Thr Ser Thr Val Pro Ser Cys Cys Cys Thr Lys
225 230 235 240

Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
245 250 255

Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu
260 265 270

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
275 280 285

Val Trp Leu Leu Val Met Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
290 295 300

Tyr Arg Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys
305 310 315 320

Leu Trp Val Tyr Ile
325

<210> 41
<211> 309
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of S15

<220>
<221> MISC_FEATURE
<222> (308)..(308)
<223> x = any amino acid

<400> 41

Pro Pro Pro Ala Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro
1 5 10 15

Leu Ser Pro Pro Leu Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn
20 25 30

Ser Thr Thr Phe His Gln Thr Leu Gln Asp Pro Arg Val Lys Gly Leu
35 40 45

Tyr Phe Pro Ala Gly Gly Ser Ser Ser Gly Thr Val Asn Pro Val Pro
50 55 60

Thr Thr Ala Ser His Ser Ser Ser Ile Phe Ser Arg Ile Gly Val Pro
65 70 75 80

Ala Leu Asn Met Glu Asn Ile Thr Ser Gly Leu Leu Gly Pro Leu Leu
85 90 95

Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro
100 105 110

Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Arg Gly Gly Thr
115 120 125

Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser
130 135 140

Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu
145 150 155 160

Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe
165 170 175

Leu Leu Ala Leu Leu Asp Cys Gln Gly Met Leu Pro Val Cys Pro Leu
180 185 190

Ile Pro Gly Ser Ser Thr Thr Ser Arg Gly Pro Cys Arg Thr Cys Thr
195 200 205

Thr Pro Ala Gln Gly Thr Ser Thr Val Pro Ser Cys Cys Cys Thr Lys
210 215 220

Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
225 230 235 240

Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu
245 250 255

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
260 265 270

Val Trp Leu Leu Val Met Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
275 280 285

Tyr Arg Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys
290 295 300

Leu Trp Val Xaa Ile
305

<210> 42
<211> 1031
<212> DNA
<213> artificial sequence

<220>
<223> Nucleotide sequence of Patient C

<400> 42
tactacaaac cttgccagca aatccgcctc ctgcctctac caatcgccag tcaggaaggc 60

agcctacccc tctgactcca cctttgagaa acactcatcc tcaggccatg cagtggaact 120

ccacaaactt ccaccgaact ctacaagatc ccagagtgaa aggcctgtat ctccctgctg 180

gtggctccag ttcaggaaca gtaaaccctg ttccgactac tgtctctcac acatcgtcaa 240

tcttatcgag gattggggac cctgcactga acatggagaa catcacatca ggattcctag 300

gacccctgct cgtgttacag gcggggtttt tcttgttgac aagaatcctc acaataccgc 360

agagtctaga ctcgtggtgg acttctctca attttctagg ggggaccacc gtgtgccttg 420

gccaaaattc gcagtcccca acctccaatc actcaccaac ctcctgtcct ccaacttgtc 480

ctggttatcg ctggatgtgt ctgcggcgtt ttatcatatt cctcttcatc ctgctgctat 540

gcctcatctt cttgttggtt cttctggact atcaaggtat gttgcccgtt tgccctctaa 600

ttccaggatc ctcaaccacc agcacgggac catgcagaac ctgcacgact cctgctcaag 660

gaacctctwt gtatccctca tgttgctgta ccaaacctwc ggmcgsaaat tgcacctgta 720

ttcccatccc atcatcctgg gctttcggaa aattcctatg ggagtgggcc tcagcccgtt 780

tctcctgact cagtttacta gtgccatttg ttcagtggtt cgtagggctt tcccccactg 840

tttggctttc agttatatgg atgatgtggt attgggggcc aggtctgtac agcatcgtga 900

ggccctttt accgctgtta ccaatttttct tttgtctctg ggtatacatt taaccccgga 960

caaaacaaaa agatggggtt actctttaca tttcatgggc tatgtcattg gatgttatgg 1020

gtcattgcca c 1031

<210> 43
<211> 316
<212> PRT
<213> artificial sequence

<220>
<223> POL Trans of Patient

<400> 43

Thr Thr Asn Leu Ala Ser Lys Ser Ala Ser Cys Leu Tyr Gln Ser Pro
1 5 10 15

Val Arg Lys Ala Ala Tyr Pro Ser Asp Ser Thr Phe Glu Lys His Ser
20 25 30

Ser Ser Gly His Ala Val Glu Leu His Lys Leu Pro Pro Asn Ser Thr
35 40 45

Arg Ser Gln Ser Glu Arg Pro Val Ser Pro Cys Trp Trp Leu Gln Phe
50 55 60

Arg Asn Ser Lys Pro Cys Ser Asp Tyr Cys Leu Ser His Ile Val Asn
65 70 75 80

Leu Ile Glu Asp Trp Gly Pro Cys Thr Glu His Gly Glu His His Ile
85 90 95

Arg Ile Pro Arg Thr Pro Ala Arg Val Thr Gly Gly Val Phe Leu Val
100 105 110

Asp Lys Asn Pro His Asn Thr Ala Glu Ser Arg Leu Val Val Asp Phe
115 120 125

Ser Gln Phe Ser Arg Gly Asp His Arg Val Pro Trp Pro Lys Phe Ala
130 135 140

Val Pro Asn Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Ser
145 150 155 160

Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His Ile Pro Leu His
165 170 175

Pro Ala Ala Met Pro His Leu Leu Val Gly Ser Ser Gly Leu Ser Arg
180 185 190

Tyr Val Ala Arg Leu Pro Ser Asn Ser Arg Ile Leu Asn His Gln His
195 200 205

Gly Thr Met Gln Asn Leu His Asp Ser Cys Ser Arg Asn Leu Tyr Phe
210 215 220

Val Ser Leu Met Leu Leu Tyr Gln Thr Phe Thr Gly Arg Lys Leu His
225 230 235 240

Leu Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro Met Gly
245 250 255

Val Gly Leu Ser Pro Phe Leu Leu Thr Gln Phe Thr Ser Ala Ile Cys
260 265 270

Ser Ala Leu Phe Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly Ile
275 280 285

His Leu Thr Pro Asp Lys Thr Lys Arg Trp Gly Tyr Ser Leu His Phe
290 295 300

Met Gly Tyr Val Ile Gly Cys Tyr Gly Ser Leu Pro
305 310 315

<210> 44
<211> 301
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Patient C

<400> 44

Leu Gln Thr Leu Pro Ala Asn Pro Pro Pro Ala Ser Thr Asn Arg Gln
1 5 10 15

Ser Gly Arg Gln Pro Thr Pro Leu Thr Pro Pro Leu Arg Asn Thr His
20 25 30

Pro Gln Ala Met Gln Trp Asn Ser Thr Asn Phe His Arg Thr Leu Gln
35 40 45

Asp Pro Arg Val Lys Gly Leu Tyr Leu Pro Ala Gly Gly Ser Ser Ser

```
     50                  55                  60

Gly Thr Val Asn Pro Val Pro Thr Thr Val Ser His Thr Ser Ser Ile
65                  70                  75                  80

Leu Ser Arg Ile Gly Asp Pro Ala Leu Asn Met Glu Asn Ile Thr Ser
                85                  90                  95

Gly Phe Leu Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu
            100                 105                 110

Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr Ser
        115                 120                 125

Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly Gln Asn Ser Gln
    130                 135                 140

Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys Pro
145                 150                 155                 160

Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile
                165                 170                 175

Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly
            180                 185                 190

Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser Thr
        195                 200                 205

Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln Gly Thr Ser Met Leu
    210                 215                 220

Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Thr Ala Ala Asn Cys Thr
225                 230                 235                 240

Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp Glu
                245                 250                 255

Trp Ala Ser Ala Arg Phe Ser Leu Ser Leu Leu Val Pro Phe Val Gln
            260                 265                 270

Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val Ile Trp Met
        275                 280                 285
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Met Trp Tyr Trp Gly Pro Gly Leu Tyr Ser Ile Val Arg
290 295 300

<210> 45
<211> 888
<212> DNA
<213> artificial sequence

<220>
<223> Nucleotide sequence of Patient D

<400> 45
tggtcacagt gccaacagtt cctcctcctg cctccaccaa tcggcagtca gggaggcagc 60

ctactcccat ctctccacct ctaagagaca gtcatcctca ggccatggtg gctcagcctg 120

ctggtggctc cagttcagga acactcaacc ctgttcccaa tattgcctct cacatctcgt 180

caatctcctt gaggactggg gaccctgcgc cgaacatgga gaacatcaca tcaggattcc 240

taggacccct gctcgtgtta caggcggggt ttttcttgtt gacaagaatc ctcacaatac 300

cgcagagtct agactcgtgg tggacttctc tcagttttct agggggatca cccgtgtgtc 360

ttggccaaaa ttcgcagtcc ccaacctcca atcactcacc aacctcctgt cctccaattt 420

gacctggtta tcgctggata tgtctgcggc gttttatcat attcctcttc atcctgccgc 480

tatgcctcat cttcttattg gttcttctgg attatcaagg tatgttgccc gtttgtcctc 540

taattccagg atccacaaca accagtgcgg gaccctgcaa aacctgcacg actcctgctc 600

aaggcaactc tatgtttccc tcatgttgct gtacaaaacc tacggatgga aattgcacct 660

gtattcccat cccatcatct tgggctttcg caaaatacct atgggagtgg gcctcagtcc 720

gtttctcttg gctcagttta ctagtgccat ttgttcagtg attcgtaggg ctttccccca 780

ctgtttggct ttcagctata ttgatgatgt ggtactgggg gccaagtctg cacaacatct 840

tgagtccctt tataccgctg ttaccaattt tcttttgtct ttgggtat 888

<210> 46
<211> 295
<212> PRT
<213> artificial sequence

<220>
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<400> 46

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Arg Glu Ala Ala Tyr Ser His Leu Ser Thr Ser Lys Arg Gln Ser Ser
20 25 30

Ser Gly His Gly Gly Ser Ala Cys Trp Trp Leu Gln Phe Arg Asn Thr
35 40 45

Gln Pro Cys Ser Gln Tyr Cys Leu Ser His Leu Val Asn Leu Leu Glu
50 55 60

Asp Trp Gly Pro Cys Ala Glu His Gly Glu His His Ile Arg Ile Pro
65 70 75 80

Arg Thr Pro Ala Arg Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn
85 90 95

Pro His Asn Thr Ala Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe
100 105 110

Ser Arg Gly Ile Thr Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn
115 120 125

Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Thr Trp Leu Ser
130 135 140

Leu Asp Met Ser Ala Ala Phe Tyr His Ile Pro Leu His Pro Ala Ala
145 150 155 160

Met Pro His Leu Leu Ile Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala
165 170 175

Arg Leu Ser Ser Asn Ser Arg Ile His Asn Asn Gln Cys Gly Thr Leu
180 185 190

Gln Asn Leu His Asp Ser Cys Ser Arg Gln Leu Tyr Val Ser Leu Met
195 200 205

Leu Leu Tyr Lys Thr Tyr Gly Trp Lys Leu His Leu Tyr Ser His Pro
210 215 220

Ile Ile Leu Gly Phe Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro
225 230 235 240

Phe Leu Leu Ala Gln Phe Thr Ser Ala Ile Cys Ser Val Ile Arg Arg
245 250 255

Ala Phe Pro His Cys Leu Ala Phe Ser Tyr Ile Asp Asp Val Val Leu
260 265 270

Gly Ala Lys Ser Ala Gln His Leu Glu Ser Leu Tyr Thr Ala Val Thr
275 280 285

Asn Phe Leu Leu Ser Leu Gly
290 295

<210> 47
<211> 293
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Patient D

<400> 47

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Gly Arg Gln Pro Thr Pro Ile Ser Pro Pro Leu Arg Asp Ser His Pro
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Gln Ala Met Val Ala Gln Pro Ala Gly Gly Ser Ser Ser Gly Thr Leu
35 40 45

Asn Pro Val Pro Asn Ile Ala Ser His Ile Ser Ser Ile Ser Leu Arg
50 55 60

Thr Gly Asp Pro Ala Pro Asn Met Glu Asn Ile Thr Ser Gly Phe Leu
65 70 75 80

Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile
85 90 95

Leu Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Ser Phe
100 105 110

Leu Gly Gly Ser Pro Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr

115 120 125

Ser Asn His Ser Pro Thr Ser Cys Pro Pro Ile Pro Gly Tyr Arg Trp
130 135 140

Ile Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Pro Leu Cys
145 150 155 160

Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val
165 170 175

Cys Pro Leu Ile Pro Gly Ser Thr Thr Thr Ser Ala Gly Pro Cys Lys
180 185 190

Thr Cys Thr Thr Pro Ala Gln Gly Asn Ser Met Phe Pro Ser Cys Cys
195 200 205

Cys Thr Lys Pro Thr Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser
210 215 220

Ser Trp Ala Phe Ala Lys Tyr Leu Trp Glu Trp Ala Ser Val Arg Phe
225 230 235 240

Ser Trp Leu Ser Leu Leu Val Pro Phe Val Gln Phe Val Gly Leu Ser
245 250 255

Pro Thr Val Trp Leu Ser Ala Ile Leu Met Met Trp Tyr Trp Gly Pro
260 265 270

Ser Leu His Asn Ile Leu Ser Pro Phe Ile Pro Leu Leu Pro Ile Phe
275 280 285

Phe Cys Leu Trp Val
290

<210> 48
<211> 591
<212> DNA
<213> artificial sequence

<220>
<223> Nucleotide sequence of Patient E

<400> 48
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ctcttcatcc tgctgctatg cctcatcttc ttattggttc ttctggatta tcaaggtatg 120

ttgcccgtct gtcctctaat tccaggatca acaacaacca gtacgggacc atgcaaaacc 180

aaaacctgca cgactcctgc tcaaggcaac tctatgtttc cctcatgttg ctgtacaaaa 240

cctacggatg gaaattgcac ctgtattccc atcccatcgt cctgggcttt cgcaaaattc 300

ctatgggagt gggcctcagt ccgtttctct tggctcagtt tactagtgcc atttgttcag 360

tggttcgtag ggctttcccc cactgtttgg ctttcagcta tatggatgat gtggtattgg 420

gggccaagtc tgtacagcat cgtgaggccc tttatacagc tgttaccaat tttcttttgt 480

ctctgggtat acatttaaac cctaacaaaa caaaaagatg gggttattcc ctaaacttca 540

tgggttacat aattggaagt tggggaacat tgccacagga tcatattgta c 591

<210> 49
<211> 186
<212> PRT
<213> artificial sequence

<220>
<223> Pol Trans of Patient E

<400> 49

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1 5 10 15

Ile Pro Leu His Pro Ala Ala Met Pro His Leu Leu Ile Gly Ser Ser
20 25 30

Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile Asn
35 40 45

Asn Asn Gln Tyr Gly Thr Met Gln Asn Gln Asn Leu His Asp Ser Cys
50 55 60

Ser Arg Gln Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr Tyr Gly
65 70 75 80

Trp Lys Leu His Leu Tyr Ser His Pro Ile Val Leu Gly Phe Arg Lys
85 90 95

Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln Phe Thr
100 105 110

Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala
115 120 125

Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val Gln His
130 135 140

Arg Glu Ala Leu Tyr Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly
145 150 155 160

Ile His Leu Asn Pro Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu Asn
165 170 175

Phe Met Gly Tyr Ile Ile Gly Ser Trp Gly
180 185

<210> 50
<211> 165
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Patient E

<400> 50

Ser Cys Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg
1 5 10 15

Phe Met Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu
20 25 30

Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro
35 40 45

Gly Ser Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Lys Thr Cys Thr
50 55 60

Thr Pro Ala Gln Gly Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys
65 70 75 80

Pro Thr Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala
85 90 95

Phe Ala Lys Phe Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu
100 105 110

Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr
115 120 125

Val Trp Leu Ser Ala Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu
130 135 140

Tyr Ser Ile Val Arg Pro Phe Ile Gln Leu Leu Pro Ile Phe Phe Cys
145 150 155 160

Leu Trp Val Tyr Ile
165

<210> 51
<211> 669
<212> DNA
<213> artificial sequence

<220>
<223> Nucleotide sequence of Patient

<400> 51
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ccacccgtgt gtcttggcca aaattcgcag tccccaacct ccaatcactc accaacctct 120

tgtcctccaa tttgtcctgg ttatcgctgg atgtgtctgc ggcgttttat catatcctc 180

ttcatcctgc tgctatgcct catcttctta ttggttcttc tggattatca aggtatgttg 240

cccgtttgtc ctctaattcc aggatccaca acaaccagta cgggaccctg caaaacctgc 300

acgactcctg ctcaaggcaa ctctatgttt ccctcatgtt gctgtacaaa acctacggat 360

ggaaattgca cmtgtattcc catcccatca tcttgggctt tcgcaaaata cctatgggag 420

tgggcctcag tccgtttctc ttggttcagt ttactagtgc catttgttca gtggttcgta 480

gggctttccc ccactgtttg gctttcagct atatggatga tattgtactg ggggccaagt 540

ctgtacaaca tcttgagtcc ctttataccg ctgttaccaa ttttcttttg tctttgggta 600

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taattggaa 669

<210> 52
<211> 181
<212> PRT
<213> artificial sequence

<220>
<223> Deduced sequence of DNA polymerase of Patient F

<220>
<221> MISC_FEATURE
<222> (83)..(83)
<223> x = any amino acid

<400> 52

Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr His
1 5 10 15

Ile Pro Leu His Pro Ala Ala Met Pro His Leu Leu Ile Gly Ser Ser
20 25 30

Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn Ser Arg Ile His
35 40 45

Asn Asn Gln Tyr Gly Thr Leu Gln Asn Leu His Asp Ser Cys Ser Arg
50 55 60

Gln Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr Tyr Gly Trp Lys
65 70 75 80

Leu His Xaa Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile Pro
85 90 95

Met Gly Val Gly Leu Ser Pro Phe Leu Leu Val Gln Phe Thr Ser Ala
100 105 110

Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe Ser
115 120 125

Tyr Met Asp Asp Ile Val Leu Gly Ala Lys Ser Val Gln His Leu Glu
130 135 140

Ser Leu Tyr Thr Ala Val Thr Asn Phe Leu Leu Ser Leu Gly Ile His
145 150 155 160

Leu Thr Pro Asn Lys Thr Lys Arg Trp Gly Tyr Ser Leu Asn Phe Met
165 170 175

Gly Tyr Val Ile Gly
180

<210> 53
<211> 160
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Patient F

<400> 53

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
1 5 10 15

Ser Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
20 25 30

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
35 40 45

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
50 55 60

Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly Asn
65 70 75 80

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
85 90 95

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Phe Ser Leu Leu Val Pro
100 105 110

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Ala
115 120 125

Ile Trp Met Ile Leu Tyr Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
130 135 140

Pro Phe Ile Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
145 150 155 160

<210> 54
<211> 554
<212> DNA
<213> artificial sequence

<220>
<223> Nucleotide sequence of Patient G

<400> 54
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cctgctgcta tgcctcatct tcttgttggt tcttctggac tatcaaggta tgttgcccgt 120

ttgtcctcta cttccaggaa catcaactac cagcacggga ccatgcaaga cctgcacgac 180

tcctgctcaa ggaacctcta tgtttccctc ttgttgctgt acaaaacctt cggacggaaa 240

ttgcacttgt attcccatcc catcgtcttg ggctttcgca agattcctat gggagtgggc 300

ctcagtccgt ttctcttggc tcartttact agtgccattt gttcagtggt tcgtagggct 360

ttcccccact gtttggcttt cagttatatt gatgatgtgg tattgggggc caagtctgta 420

caacatcttg aatccctttt tacctctatt accaattttc ttatgtcttt gggtatacat 480

ttaaacccta agaaaaccaa acgttggggc tactccctta acttcatggg atatgtaatt 540

ggaagttggg gtac 554

<210> 55
<211> 184
<212> PRT
<213> artificial sequence

<220>
<223> Deduced sequence of DNA polymerase of Patient G

<400> 55

Ser Asn Leu Ser Trp Val Ser Leu Asp Val Ser Ala Ala Phe Tyr His
1 5 10 15

Ile Pro Leu His Pro Ala Ala Met Pro His Leu Leu Val Gly Ser Ser
20 25 30

Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Thr Ser Arg Asn Ile
35 40 45

Asn Tyr Gln His Gly Thr Met Gln Asp Leu His Asp Ser Cys Ser Arg
50 55 60

Asn Leu Tyr Val Ser Leu Leu Leu Leu Tyr Lys Thr Phe Gly Arg Lys
65 70 75 80

Leu His Leu Tyr Ser His Pro Ile Val Leu Gly Phe Arg Lys Ile Pro
85 90 95

Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln Phe Thr Ser Ala
100 105 110

Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys Leu Ala Phe Ser
115 120 125

Tyr Ile Asp Asp Val Val Leu Gly Ala Lys Ser Val Gln His Leu Glu
130 135 140

Ser Leu Phe Thr Ser Ile Thr Asn Phe Leu Met Ser Leu Gly Ile His
145 150 155 160

Leu Asn Pro Lys Lys Thr Lys Arg Trp Gly Tyr Ser Leu Asn Phe Met
165 170 175

Gly Tyr Val Ile Gly Ser Trp Gly
180

<210> 56
<211> 160
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Patient G

<220>
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<222> (108)..(108)
<223> x = any amino acid

<400> 56

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Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
20 25 30

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Thr Ser
35 40 45

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
50 55 60

Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn
65 70 75 80

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Arg Phe Leu
85 90 95

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Xaa Leu Leu Val Pro
100 105 110

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
115 120 125

Ile Leu Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Asn Ile Leu Asn
130 135 140

Pro Phe Leu Pro Leu Leu Pro Ile Phe Leu Cys Leu Trp Val Tyr Ile
145 150 155 160

<210> 57
<211> 1045
<212> DNA
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<220>
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aaactctgcw agatcccaga gtgagaggcc tgtatttccc tgctggtggc tccagttcag 180

gaacagtaaa ccctgttccg acttctgtct ctcacacatc gtcaatcttc tcgaggattg 240

gggwccctgc gctgaacatg gagaacatca catcaggatt cctaggaccc ctgctcgtgt 300

tacaggcggg gtttttcttg ttgacaagaa tcctcacaat accgcagagt ctagactcgt 360

ggtggacttc tctcaatttt ctaggggaa ctaccgtgtg tcttggccaa aattcgcagt 420

tcccaacctc caatcactca ccaacctcct gtcctccaac ttgwcctggt tatcgctgga 480

tgtrtctgcg gcgttttatc atcttcctct tcatcctgct gctatgcctc atcttcttgt 540

tggttcttct ggactatcaa ggtatgttgc ccgtttgtcc tctarttcca ggatcttcaa 600

ccaccagcac gggaccatgc agaacctgca cgactcctgc tcaaggaamc tctatgaatc 660

cctcctgttg ctgtaccaaa ccttcggacg gaaattgcac ctgtattccc atcccatcat 720

cctgggcttt cggaaaattc ctatgggagt gggcctcagc ccgtttctcc tgrctcagtt 780

tactagtgcc atttgttcag tggttcgtag ggctttcccc cactgtttgg ctttcagtta 840

tatggatgat gtggtattgg gggccaagtc tgtaymgcat cttragtccc ttttttaccgc 900

tgttaccaat tttcttttgt ctytgggtat acatttaaac cctmacaaaa caaaaagatg 960

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tcacatcaga cagaaaatca aagaa 1045

<210> 58
<211> 348
<212> PRT
<213> artificial sequence

<220>
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<223> x = any amino acid

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<223> x = any amino acid

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<222> (315)..(315)
<223> x = any amino acid

<400> 58

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Tyr Pro Ser Val Ser Thr Phe Xaa Lys His Ser Ser Ser Gly His Ala
20 25 30

Val Glu Leu His Asn Leu Pro Pro Asn Ser Ala Arg Ser Gln Ser Glu
35 40 45

Arg Pro Val Phe Pro Cys Trp Trp Leu Gln Phe Arg Asn Ser Lys Pro
50 55 60

Cys Ser Asp Phe Cys Leu Ser His Ile Val Asn Leu Leu Glu Asp Trp
65 70 75 80

Gly Pro Cys Ala Glu His Gly Glu His His Ile Arg Ile Pro Arg Thr
85 90 95

Pro Ala Arg Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His
100 105 110

Asn Thr Ala Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg
115 120 125

Gly Asn Tyr Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln
130 135 140

Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Xaa Trp Leu Ser Leu Asp
145 150 155 160

Val Ser Ala Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro
165 170 175

His Leu Leu Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu
180 185 190

Ser Ser Xaa Ser Arg Ile Phe Asn His Gln His Gly Thr Met Gln Asn
195 200 205

Leu His Asp Ser Cys Ser Arg Xaa Leu Tyr Glu Ser Leu Leu Leu Leu
210 215 220

Tyr Gln Thr Phe Gly Arg Lys Leu His Leu Tyr Ser His Pro Ile Ile
225 230 235 240

Leu Gly Phe Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu
245 250 255

Leu Xaa Gln Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe
260 265 270

Pro His Cys Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala
275 280 285

Lys Ser Val Xaa His Leu Xaa Ser Leu Phe Thr Ala Val Thr Asn Phe
290 295 300

Leu Leu Ser Leu Gly Ile His Leu Asn Pro Xaa Lys Thr Lys Arg Trp
305 310 315 320

Gly Tyr Ser Leu His Phe Met Gly Tyr Val Ile Gly Cys Tyr Gly Ser
325 330 335

Leu Pro Gln Asp His Ile Arg Gln Lys Ile Lys Glu
340 345

<210> 59
<211> 311
<212> PRT
<213> artificial sequence

<220>
<223> HBsAg Trans of Patient H

<220>
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<222> (24)..(24)

<223> x = any amino acid

<220>
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<220>
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<223> x = any amino acid

<220>
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<222> (154)..(154)
<223> x = any amino acid

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<222> (161)..(161)
<223> x = any amino acid

<220>
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<223> x = any amino acid

<220>
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<222> (216)..(216)
<223> x = any amino acid

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<222> (257)..(257)
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<222> (292)..(292)
<223> x = any amino acid

<400> 59

Ala Asn Pro Pro Pro Ala Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro
1 5 10 15

Thr Pro Leu Ser Pro Pro Leu Xaa Asn Thr His Pro Gln Ala Met Gln
20 25 30

Trp Asn Ser Thr Thr Phe His Gln Thr Leu Xaa Asp Pro Arg Val Arg
35 40 45

Gly Leu Tyr Phe Pro Ala Gly Gly Ser Ser Ser Gly Thr Val Asn Pro
50 55 60

Val Pro Thr Ser Val Ser His Thr Ser Ser Ile Phe Ser Arg Ile Gly
65 70 75 80

Xaa Pro Ala Leu Asn Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro
85 90 95

Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr
100 105 110

Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly
115 120 125

Gly Thr Thr Val Cys Leu Gly Gln Asn Ser Gln Phe Pro Thr Ser Asn
130 135 140

His Ser Pro Thr Ser Cys Pro Pro Thr Xaa Pro Gly Tyr Arg Trp Met
145 150 155 160

Xaa Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu
165 170 175

Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys
180 185 190

Pro Leu Xaa Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr
195 200 205

Cys Thr Thr Pro Ala Gln Gly Xaa Ser Met Asn Pro Ser Cys Cys Cys
210 215 220

Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser
225 230 235 240

Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser

245 250 255

Xaa Leu Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser
260 265 270

Pro Thr Val Trp Leu Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro
275 280 285

Ser Leu Tyr Xaa Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe
290 295 300

Phe Cys Leu Trp Val Tyr Ile
305 310

a

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00432

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12N7/00, 15/01, 15/36, 15/51, C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, MEDLINE, CA. KEYWORDS: Hepatitis B/Nucleotide/Nucleoside/Resistan?/Muta?/Adefovir/ Lamivudine/Tenofivir/Thiacydidine/HBsAg/Polymerase and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/94559 (Melbourne Health) 13 December 2001 Claims, p.12-19, p.22 line 25- p.24 line 25, and p. 25 line 30 - p. 26 line 9	1-125, 169-202
X	WO 01/57244 (Melbourne Health) 9 August 2001 Claims, Table 1, p. 17 line 7 - p. 21 line 12 and p. 25 line 16 - p. 33 line 2	1-17, 19-74, 80, 86, 95, 106, 119, 123-127

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 May 2003	06 JUN 2003
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer ALISTAIR BESTOW Telephone No : (02) 6283 2450

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00432

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : **126, 159, 160 (completely), 161-168 (partially)**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The claims are not restricted to the technical features of the invention, namely hepatitis B variants, methods of using these variants and products of these methods.
Claims 161-168 have only been searched in so far as they relate to HBV nucleic acid or peptides sequences, antibodies, ribozymes and antisense that are capable of inhibiting the variant HBVs of the inventiont

3. ☐ Claims Nos :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for summary inventions.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00432

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/21317 (Western Health Care Network) 22 May 1998 Claims, p. 4 line 5 - p. 7 line 12 and p. 8 line 28 - p. 11 line 20,	1-17, 19-37, 39, 40, 42-50, 54, 55, 58-74, 76-78, 80-90, 94, 95, 98, 100-114, 118, 119, 122, 170-178, 180-182, 184-186, 190, 194-202
X	Yeh, C. et al., 2000, *Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy*, Hepatology, 31:1318-1325 See whole document	1-17, 19-37, 39, 40, 49, 50, 54, 55, 58-74, 76, 77, 78, 82, 85, 86, 89, 90, 94, 95, 98, 100-102, 106, 109, 113, 114, 118, 119, 122, 127-129
X	Seigneres, B. et al., 2000, *Evolution of Hepatitis B virus polymerase gene sequence during famciclovir therapy for chronic hepatitis B*, Journal of Infectious Diseases, 181:1221-33 See whole document	1-17, 19-38, 42, 50, 54, 57, 58-74, 80, 85, 86, 90, 97, 104, 109, 110, 114, 118, 121, 122, 127-129
X	Ogata, N. et al., 1999, *Novel patterns of amino acid mutations in the hepatitis b virus polymerase in association with resistance to lamivudine therapy in japanese patients with chronic hepatitis B*, Journal of Medical Virology, 59:270-276 See whole document	1-17, 19-37, 43, 59-74, 81, 105
X	Bock, C. et al., 2002(February), *Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation*, Gastroenterology, 122:264-273 See whole document	1-17, 19-37, 39, 44, 54, 55, 58-74, 83, 94, 95, 98, 100, 107, 118, 119, 122

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00432

C (Continuation) *DOCUMENTS CONSIDERED TO BE RELEVANT*

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Cane, P. et al., 1999, *Analysis of hepatitis B virus quasispecies changes during the emergence and reversion of lamivudine resistance in liver transplantation*, Antiviral Therapy, 4:7-14 See whole document	1-17, 19-37, 39, 43, 45, 49, 50, 55, 58-74, 81, 84, 86, 89, 90, 94, 95, 98, 100, 102, 108, 110, 113, 114, 118,119, 122

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No II:

Invention 1: Claims 1- 20, 38, 59-75, 99, 123-125, 127, 130-134, 170, 178, 179, 195 (all partially) relating to an isolated Hepatitis B virus (HBV) variant comprising a mutation in a gene encoding DNA polymerase resulting in an amino acid addition, substitution and/or deletion at amino acid position rt21 of the HBV DNA polymerase, the use of these variants, and methods for determining whether a HBV variant exhibits decrease sensitivity to anti-viral agents or reduced interactivity to an antibody to HBV surface antigen.

Invention 2: 1- 20, 38, 59-75, 77, 99, 101, 123-125, 127, 130-134, 136, 170, 178, 179, 181, 195 (all partially) relating to an isolated HBV variant comprising a mutation in a gene encoding DNA polymerase resulting in an amino acid addition, substitution and/or deletion at amino acid position rt122 of the HBV DNA polymerase, the use of these variants, and methods for determining whether a HBV variant exhibits decrease sensitivity to anti-viral agents or reduced interactivity to an antibody to HBV surface antigen.

Inventions 3-37: relating to HBV variants comprising a mutation in a gene encoding DNA polymerase and/or HBsAg resulting in an amino acid addition, substitution and/or deletion at amino acid positions rt124, rt28, rt130 , etc, rt251 or the equivalent position in the overlapping HBsAg, the use of these variants, and methods for determining whether a HBV variant exhibits decrease sensitivity to anti-viral agents or reduced interactivity to an antibody to HBV surface antigen.

The common feature linking the group of inventions resides in the elucidation of the link between reduced sensitivity to anti-HBV agents such as ADV, LMV, FTC and TFV and mutation of the nucleotide sequence encoding HBV DNA polymerase or the overlapping S gene (HBsAg). However, this is already known in the art (see Chen et al., 1999, Human hepatitis B virus mutants: significance of molecular changes, FEBS Letters, 453:237-242, and citations below), as such the claims relate to multiple inventions, *a posteriori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU03/00432

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	200194559	AU	20008109	AU	200163672	EP	1297109
WO	200157244	AU	200131415	EP	1257661		
WO	9821317	AU	37628/97	EP	964916	US	6555311

END OF ANNEX